Laser Burn Intensity and the Risk for Choroidal Neovascularization in the CNVPT Fellow Eye Study

Richard S. Kaiser, MD; Jeffrey W. Berger, MD, PhD†; Maureen G. Maguire, PhD; Allen C. Ho, MD; Noreen B. Javornik, MS; and the Choroidal Neovascularization Prevention Trial (CNVPT) Study Group

Objective: To explore the relationship between laser burn intensity and the subsequent risk for development of choroidal neovascularization (CNV) in eyes assigned to the treatment group of the Fellow Eye Study (FES) of the Choroidal Neovascularization Prevention Trial (CNVPT), using computerized methods for laser burn quantitation, and to examine the association between laser burn intensity and (1) drusen reduction and (2) visual acuity.

Methods: Color fundus images before and immediately after laser treatment in the CNVPT FES were available for 53 of 59 eyes. Prelaser and postlaser treatment images were analyzed using custom-developed computer software, allowing for laser burn identification and quantitation. As measures of laser burn intensity, we derived integrated burn rating (IBR) (the integral of the normalized intensity difference divided by the burn pixels), and the maximum burn intensity (MAX). We identified CNV using fluorescein angiography. A Cox proportional hazards model was fit to the time to development of CNV. Baseline and 6-month color photographs were used to determine reduction in drusen. Visual acuity was measured using a standardized protocol.

Results: The IBR and MAX spanned 4.5 logarithm units. After adjusting for smoking history and predominant drusen size, the risk ratio for CNV per logarithm unit of increasing laser burn intensity for each measure was 2.0 ($P = .05$) for MAX and 1.7 ($P = .07$) for IBR. When patients were divided into high- and low-intensity treatment groups of equal size, the high-intensity group had more drusen reduction (57% vs 32%; $P = .14$). There was no effect of laser intensity on change in visual acuity at 6 months.

Conclusion: Higher-intensity prophylactic laser applications appear to be associated with a greater risk for development of CNV and with more extensive drusen reduction.

Arch Ophthalmol. 2001;119:826-832
PATIENTS AND METHODS

PATIENT SELECTION AND TREATMENT

The detailed eligibility criteria for the CNVPT have been reported previously. Enrollment of patients began in October 1994. Patients enrolled in the FES of the CNVPT were required to have CNV secondary to AMD in 1 eye and 10 or more large (>63 µm) drusen in the contralateral (fellow) eye. Fluorescein angiography was performed to document the absence of CNV in the study eye at the time of enrollment. Each patient signed a consent statement that had been approved by the institutional review board associated with the local CNVPT clinical center. The 59 eyes randomly assigned to laser treatment were treated according to 1 of the 3 laser treatment protocols used in the CNVPT. Most eyes (49 [83%] of 59) were treated with the laser-20 protocol consisting of 20 laser burns applied using a 100-µm spot size. Burns were placed in 3 concentric rows subtending 180° in the temporal macula, with the innermost row at least 750 µm from the center of the fovea. The intensity of the burn was specified as gray-white. An additional 9 eyes (15%) were treated with the laser-24 protocol consisting of 24 laser burns applied using a 100-µm spot size. Burns were placed in 2 concentric circles of 12 evenly spaced burns, with the innermost circle at least 750 µm from the center of the fovea. Again, intensity was specified as gray-white. One eye (2%) was treated with the laser-06 protocol consisting of 6 laser burns applied using a 100-µm spot size. Burns were placed in a circle of 6 evenly spaced burns approximately 1000 µm from the center of the fovea. Intensity was specified as gray-white.

The CNVPT protocol specified that eyes without a 50% or greater drusen reduction by 6 months were to receive a second treatment. However, because of the suspension in treatment recommended by the Data and Safety Monitoring Committee in December 1996, some eyes that did not have a 50% reduction in drusen area were not re-treated. Twenty-two (45%) of the 49 eyes assigned to the laser-20 protocol received additional treatment on the nasal side of the fovea, in a mirror image of the pattern of the initial treatment applied on the temporal side. None of the eyes assigned to the laser-24 or laser-06 treatment protocol were eligible for re-treatment because they had not yet reached 6 months of follow-up by December 1996.

RESULTS

Laser spots were identified, and the intensity of the laser burns were quantified using custom-developed, computer-assisted techniques. The 35-mm color fundus photographs of CNVPT FES eyes acquired immediately before and after laser prophylaxis application were digitized at 1000 dots per inch using a slide scanner (Microtek 35T; Microtek, Los Angeles, Calif). In re-treated eyes, measurements were performed for both treatments.

Laser spot identification followed (1) geometric normalization of pretreatment and posttreatment photographs to correct for translational, rotational, magnification, and warp-related image misalignment; (2) radiometric normalization to correct for brightness differences between the 2 images; and (3) image subtraction to detect often subtle retinal color change after laser application.

Several methods for image registration were explored, and it was determined that highly accurate detection of image change requires precise image registration incorporating nonglobal, warping transformations. This was accomplished with polynomial warping algorithms described previously. Radiometric normalization was accomplished using the method previously described by Algazi et al. Briefly, the mean and SD of the pixel values in the region of interest containing the laser burns in the pretreatment and posttreatment photographs were equalized. Without radiometric normalization, false-positive areas of change would be identified.

After geometric and radiometric normalization, the pretreatment image was subtracted from the posttreatment image. Extraneous, isolated pixels, which would otherwise be identified as laser spots, were eliminated by noting that a laser spot must subtend some minimum area on the retina corresponding to an area of contiguous pixels. The minimum number of contiguous pixels occupied by a laser burn was set to 10 (each pixel representing a square approximately 6 × 6 µm), with this value adjustable by a supervisor in real time.

A threshold value was selected arbitrarily to identify the magnitude of the normalized brightness difference between the 2 images corresponding to laser spots. This value was correlated with CNV incidence, drusen reduction, and change in visual acuity.

Laser burn quantitation was performed for 53 (90%) of the 59 patients assigned to treatment in the FES. One patient died within 3 months of enrollment and could not contribute data on the development of CNV, and 5 patients had missing pretreatment or posttreatment photographs, which precluded computer-assisted laser burn quantitation. Inspection of the distribution of the IBR among patients and of the maximum-intensity burn rating (MAX) showed marked skewness. Therefore, the logarithm of the values was used for all further analyses.

COMPUTER-ASSISTED LASER BURN EVALUATION

Laser spots were identified, and the intensity of the laser burns were quantified using custom-developed, computer-assisted techniques. The 35-mm color fundus photographs of CNVPT FES eyes acquired immediately before and after laser prophylaxis application were digitized at 1000 dots per inch using a slide scanner (Microtek 35T; Microtek, Los Angeles, Calif). In re-treated eyes, measurements were performed for both treatments.

Laser spot identification followed (1) geometric normalization of pretreatment and posttreatment photographs to correct for translational, rotational, magnification, and warp-related image misalignment; (2) radiometric normalization to correct for brightness differences between the 2 images; and (3) image subtraction to detect often subtle retinal color change after laser application.

Several methods for image registration were explored, and it was determined that highly accurate detection of image change requires precise image registration incorporating nonglobal, warping transformations. This was accomplished with polynomial warping algorithms described previously. Radiometric normalization was accomplished using the method previously described by Algazi et al. Briefly, the mean and SD of the pixel values in the region of interest containing the laser burns in the pretreatment and posttreatment photographs were equalized. Without radiometric normalization, false-positive areas of change would be identified.

After geometric and radiometric normalization, the pretreatment image was subtracted from the posttreatment image. Extraneous, isolated pixels, which would otherwise be identified as laser spots, were eliminated by noting that a laser spot must subtend some minimum area on the retina corresponding to an area of contiguous pixels. The minimum number of contiguous pixels occupied by a laser burn was set to 10 (each pixel representing a square approximately 6 × 6 µm), with this value adjustable by a supervisor in real time.

A threshold value was selected arbitrarily to identify the magnitude of the normalized brightness difference between the 2 images corresponding to laser spots. This value was correlated with CNV incidence, drusen reduction, and change in visual acuity.
The range of the natural logarithm of the IBR (log[IBR]) from the initial treatment was from 0 to 4.56 (mean, 2.60; SD, 1.14). The range of the natural logarithm of the MAX (log[MAX]) from the initial treatment was from 0 to 5.31 (mean, 3.30; SD, 0.97). Among the 20 eyes that were re-treated under the laser-20 protocol, the distributions of the log[IBR] and log[MAX] from the second treatment were similar to the distributions at the initial treatment (r=0.81 for log[IBR] and r=0.82 for log[MAX]). Both measures of intensity, log[IBR] and log[MAX], were also correlated (r=0.73 for the initial treatment and r=0.75 for the second treatment). Thus, in general, eyes with a lower IBR also had a lower MAX; eyes with 1 or 2 burns that were much more intense than the remainder of the burns in the eye were relatively rare.

Each eye was also graded subjectively for reduction relative to baseline of 50% or more in the area of drusen within 3000 µm of the foveal center.

Choroidal neovascularization was defined by the presence of dye leakage on fluorescein angiography. Fluorescein angiograms were performed annually and when signs or symptoms of CNV were present.

Vision was assessed at baseline and at each follow-up visit by certified CNVPT examiners using standardized methods for refraction and distance visual acuity measurement. The CNVPT protocol for these procedures was adapted from the Age-Related Eye Disease Study with the modification that the measurement of visual acuity began at a distance of 3.2 m rather than 4.0 m. Visual acuity was scored as the number of letters read correctly.

DATA ANALYSIS

Patient age, sex, history of cigarette smoking, current use of aspirin, presence of definite hypertension, current use of vitamins and/or dietary supplements, and initial visual acuity score obtained during the patient’s initial CNVPT visit were noted. Grading of the photographs from the initial visit were summarized as the number of sectors with each of the following fundus characteristics: focal hyperpigmentation, presence of more than 20 drusen measuring greater than 63 µm, presence of drusen measuring greater than 250 µm, predominant drusen size, and confluent drusen. All of these patient and ocular factors were considered as potentially confounding in the statistical analysis of each outcome.

Factors influencing the time to development of CNV were assessed using the Cox proportional hazards model with time-dependent covariates to incorporate the measures of laser burn intensity applied at 6 months for some patients. Kaplan-Meier estimates were used to estimate the incidence of CNV in subgroups of patients on the basis of the level of laser burn intensity. Factors influencing the reduction of drusen by 50% or more were assessed using logistic regression analyses, with a robust variance estimator when considering multiple areas of the macula of each patient. Factors influencing change in visual acuity were assessed using multiple linear regression.
session (P > .80). Therefore, the influence of treatment intensity was summarized using only the measures from the initial treatment.

After adjusting for history of cigarette smoking and the area covered by predominantly larger drusen, the risk ratio for the development of CNV associated with a 1-logarithm unit increase in the IBR was 1.69 (95% confidence interval [CI], 0.96-2.96; P = .07). After adjusting
for the same covariates, the risk ratio associated with a 1-logarithm unit increase in the MAX was 1.98 (95% CI, 1.01-3.87; \( P = .05 \)). For the purpose of illustrating the magnitude of the effect of treatment intensity on the development of CNV, the patients were divided into low- and high-intensity subgroups by using the median value of the IBR as a dividing point. Choroidal neovascularization developed in more eyes in the high-intensity group compared with only 32% (7/22) in the low-intensity group who had less drusen in 5 of those who did not undergo evaluation, and 3 did not have interpretable photographs at the 6-month visit. Analyses using logistic regression did not identify any risk factors from among the baseline characteristics for reduction of drusen by 50% or more at 6 months. The odds ratio for drusen reduction associated with a 1-logarithm unit increase in the IBR was 1.59 (95% CI, 0.89-2.84; \( P = .12 \)). The odds ratio associated with a 1-logarithm unit increase in the MAX was 1.21 (95% CI, 0.66-2.22; \( P = .54 \)). When the patients were divided into groups based on the median IBR value as the dividing point, 57% (13/23) of the high-intensity group compared with only 32% (7/22) of the low-intensity group had a 50% drusen reduction at 6 months (Figure 4). The additional treatment at 6 months mandated by the laser-20 protocol when a 50% reduction in drusen had not been achieved precluded analyses of drusen reduction at later follow-ups.

The influence of laser burn intensity was explored further by comparing the proportion of patients in the high- and low-intensity groups who had less drusen in each of 4 sectors of the macula (Table). Only the 36 patients assigned to the laser-20 protocol, exclusively involving treatment temporal to the fovea, were included in the analysis. Any degree of reduction of drusen at 6 months compared with baseline—not just 50% or greater—was considered a reduction. Overall, there was a smaller proportion of patients with drusen reduction in the sector more than 1500 µm nasal to the foveal center, ie, the sector farthest from the initial treatment. In all sectors, the high-intensity group had the larger proportion of eyes with less drusen (\( P = .003 \)).

**VISUAL ACUITY**

Fifty (94%) of the 53 patients had visual acuity data available at the 6-month visit. Analyses using linear regression showed that patients with better initial visual acuity lost less visual acuity. Patients having more focal hyperpigmentation lost more visual acuity between the initial visit and the 6-month visit. After adjustment for both factors, the mean change in visual acuity was 0.14 lines worse per logarithm unit increase in IBR (95% CI, −0.29 to 0.12; \( P = .40 \)) and 0.07 lines worse per logarithm unit increase in MAX (95% CI, −1.49 to 0.79; \( P = .55 \)). When both groups were again divided based on the IBR, the high- and low-intensity groups had fairly similar distributions of change in visual acuity, with the high-intensity group having a slightly higher proportion of eyes with decreases in visual acuity (Figure 5). None of the differences in change in visual acuity based on laser burn intensity approached statistical significance.

Although major efforts are ongoing around the world toward the development of treatment strategies for late AMD, effective prophylactic strategies may contribute even more significantly toward prevention of blindness and preservation of visual function in patients at risk for visual loss due to late AMD.20

Deficiencies in our understanding of the precise pathophysiological features for the development of the vision-limiting sequelae of late AMD have limited exploration toward the development of prophylaxis strategies. However, early observations noting that macular laser application may promote drusen resorption have stimulated investigations toward evaluation of low-intensity laser treatment as a prophylaxis strategy for eyes with high-risk drusen.9,7,11

Early studies have used a wide range of laser wavelengths and ophthalmoscopic burn end points, with treatment strat-
egies involving direct treatment of drusen and treatments specifically designed to avoid drusen.21 Although not studied quantitatively, most studies have observed at least some drusen resolution after laser application.

The ophthalmoscopic end point of various treatments has varied from gray-white burns in some studies to subophthalmoscopically visible burns (burns not visible on examination) in others. Of course, intense laser burns may rupture the Bruch’s membrane and promote development of CNV, whereas ultramild laser burns may deliver insufficient photons to confer harmful or beneficial effects.

Although a treatment standard photograph was provided to the CNVPT treating ophthalmologists, laser burn intensity varied considerably among treated eyes in the CNVPT. Therefore, following the development of appropriate tools permitting laser burn quantitation, we were able to explore explicitly the influence of laser burn intensity on the risk for CNV, the likelihood of drusen resolution, and associations with a change in visual acuity.

This study represents, to our knowledge, the first quantitative exploration of drusen resolution as a function of burn magnitude. Our study demonstrates quantitatively that more intense burns were more likely to promote drusen resolution when compared with lighter burns. Moreover, drusen more proximal to the treatment burns resolved more quickly than those more distal to the treatment burns. These results agree with those of Friberg,11 who reported that 810-nm laser burns that were visible immediately after laser application were more likely to stimulate early drusen resolution (6-12 months), compared with 810-nm laser burns that did not affect a retinal or retinal pigment epithelial color change.

Our data demonstrate that laser burn intensity is related to the risk for development of CNV. As measured by 2 variables, IBR and MAX, intense treatment can increase the risk for development of CNV. One might speculate that laser-induced mechanical injury of the Bruch’s membrane may allow for development and progression of CNV. However, it is perhaps more likely that the local tissue injury and repair process may result in the production of growth factors and angiogenic molecules facilitating the development of CNV.

Available data relevant to the mechanism of action for laser-induced drusen resolution are extremely limited. In the most pertinent study, Duvall and Tso22 observed choriocapillaris-derived macrophages mediating drusen resolution in a monkey model. The challenge, therefore, is to deliver sufficient energy to promote a protective effect (which may be related to, or independent of, drusen resolution), while limiting the risk for stimulation of CNV. These first quantitative data suggest that among treated eyes in the CNVPT FES, CNV was more likely to develop in those with the most intense laser applications. Therefore, for visible laser wavelengths, it appears that a gray-white end point may be too intense. Accordingly, the Complications of AMD Prevention Trial, a randomized, prospective, multicentered study supported by the National Eye Institute, was designed to apply barely visible laser burns to 1 eye of patients with bilateral, high-risk drusen.

One might be tempted to extrapolate these data to prophylaxis trials using other wavelengths. However, differences among laser–tissue interactions as a function of wavelength preclude valid extrapolation. For infrared diode laser application at 810 nm, for example, far more energy must be delivered to mediate an equivalent temperature rise at the retinal pigment epithelium and an equivalent ophthalmoscopic end point.23,24 Therefore, ophthalmoscopic end point is not a comparable feature. For burns of equivalent visibility, far more energy is deposited in the subjacent choriocapillaris and choroid for infrared laser burns; therefore it might be predicted that infrared laser burns must be much less visible than green laser burns to reduce the potential for CNV stimulation. The relevance of wavelength to the potential for prophylactic effect and for stimulating CNV is not known.

### Laser Burn Location in the Macula

<table>
<thead>
<tr>
<th>Intensity</th>
<th>Drusen Reduction</th>
<th>Temporal to Fovea</th>
<th>Nasal to Fovea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;1500</td>
<td>1500-3000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;1500</td>
<td>1500-3000</td>
</tr>
<tr>
<td>Low</td>
<td>Yes</td>
<td>13 (72)</td>
<td>10 (56)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11 (61)</td>
<td>3 (17)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>5 (28)</td>
<td>8 (44)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 (39)</td>
<td>15 (83)</td>
</tr>
<tr>
<td>High</td>
<td>Yes</td>
<td>17 (94)</td>
<td>15 (83)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13 (72)</td>
<td>8 (44)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>1 (6)</td>
<td>3 (17)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 (28)</td>
<td>10 (56)</td>
</tr>
</tbody>
</table>

* Data are given as number (percentage) of patients.
CONCLUSIONS

These data shed light on 2 simultaneous processes dependent to some extent on burn intensity. Although higher-intensity laser treatment is associated with a greater reduction of drusen, it is also associated with an early increased risk for development of CNV. Maintaining control of these pathways is crucial to establish future prophylactic laser treatments for AMD. In fact, the results of this report warn against the use of laser burns of moderate intensity for the prophylaxis of late AMD. The outcomes reported in this trial have been crucial for the development of future protocols for applying laser burn prophylaxis to eyes with high-risk characteristics for development of late AMD. The recently initiated Complications of AMD Prevention Trial was designed to apply barely visible laser burns to 1 eye of patients with bilateral, high-risk drusen, to preserve visual function and achieve drusen resolution without the short-term increased risk for development of CNV.

Accepted for publication December 21, 2000.

Supported by a Career Development Award (Dr Berger) and an unrestricted grant to the University of Pennsylvania, Philadelphia, from Research to Prevent Blindness, New York, NY; Mackall Trust, New York; Scheie Eye Institute Macular Degeneration Research Fund, Philadelphia; grant K-080374 (Dr Berger) and grant Y11275 (CNVPT Study Group) from the National Institutes of Health, Bethesda, Md; and American Health Assistance Foundation, Rockville, Md (Dr Berger).

Presented in part at the annual meeting of the Association for Research in Vision and Ophthalmology, Fort Lauderdale, Fla, May 11, 1999.

None of the authors have a proprietary interest in this study.

Corresponding author and reprints: Maureen G. Maguire, PhD, Scheie Eye Institute, University of Pennsylvania, 51 N 39th St, Philadelphia, PA 19104 (e-mail: maguirem@mail.med.upenn.edu).

REFERENCES