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

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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.

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CHOROIDAL neovascularization (CNV) and geographic atrophy secondary to age-related macular degeneration (AMD) remain the leading causes of irreversible, severe visual loss in the United States and many developed countries. The absence of effective treatment for most patients in whom these vision-threatening, late stages of AMD develop and the fact that many patients have lost substantial visual function by the time of diagnosis have prompted a search for prophylactic strategies for patients at high risk for development of vision-threatening late AMD.

The presence of large drusen in the central macula has been shown to be a strong risk factor for the development of CNV.1-4 Stimulated by observations that macular laser application may promote drusen resolution, several randomized clinical trials have been initiated to evaluate whether laser treatment can reduce visual loss due to the development of the late stages of AMD.5-11 To date, none of these trials have provided definitive evidence of long-term treatment benefit or harm.

The Choroidal Neovascularization Prevention Trial (CNVPT) is a pilot trial addressing the effects of laser prophylaxis for eyes at risk for visual loss due to late AMD. The CNVPT was designed to assess the short-term safety of low-intensity laser application, to refine the procedures for treatment, and to assess the feasibility and logistical considerations relevant to a larger, more definitive trial.

 Patients with bilateral macular drusen were enrolled in the Bilateral Drusen Study. Patients with neovascular AMD in 1 eye and macular drusen in the fellow eye were enrolled in the Fellow Eye Study (FES). Early results demonstrated dru-
PATIENTS AND METHODS

PATIENT SELECTION AND TREATMENT

The detailed eligibility criteria for the CNVPT have been reported previously.9 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.10 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.

sensor resolution in treated eyes from both studies and a modest improvement in visual acuity in those eyes sustaining substantial drusen resolution.13 However, early data also suggested an increased incidence of CNV in treated eyes enrolled in the FES. Accordingly, to comply with recommendations of the CNVPT Data Safety and Monitoring Committee in December 1996, some eyes that did 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.13,14

Radiometric normalization was accomplished using the method previously described by Algazi et al.13 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 correlated with CNV incidence, drusen reduction, and change in visual acuity.

RESULTS

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.

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threshold is adjustable in real time in a graphical user interface designed to facilitate laser spot identification. The software allows the supervisor to flicker between the aligned pretreatment and posttreatment photographs to facilitate change detection and spot identification. Pixels erroneously identified as laser spots can be removed, whereas laser spots apparent to the supervisor but not detected by the software can be added with the use of a mouse. Initial gradings were performed by a senior ophthalmology resident (R.S.K.) and reviewed by a retinal specialist (J.W.B.). Both supervisors were masked with respect to CNV development, drusen reduction, and visual acuity.

The following 3 measures of the laser treatment were calculated: (1) the total number of pixels subtended by laser burn, (2) the magnitude of the maximum-intensity laser burn as calculated by means of the maximum value of the normalized brightness difference among all identified pixels, and (3) an integrated burn rating (IBR) (ie, the integral divided by the laser burn pixels) of the normalized intensity difference between the images (Figure 1 and Figure 2).

ASSESSMENT OF BASELINE CHARACTERISTICS, CNV, DRUSEN REDUCTION, AND VISUAL ACUITY

Stereoscopic color fundus photographs were taken before and after laser treatment and at the 6-month and 1-year follow-up visits. The photographs were examined by the staff of the CNVPT Reading Center to assess the number of visible burns and burn intensity relative to a photographic standard. Baseline drusen characteristics noted included the number of drusen greater than 63 µm in diameter, area covered with drusen, confluence of drusen, size of the largest drusen, predominant drusen size, focal hyperpigmentation, and presence of geographic atrophy for each of 4 sectors of the macula, as per CNVPT protocol.8 Sectors were defined as temporal or nasal to the fovea and as within 1500 µm of the foveal center or between 1500 and 3000 µm of the foveal center, as measured using Wisconsin templates.8 Comparisons between follow-up and baseline photographs were performed to determine whether the total area of drusen was the same, more, or less than at baseline and whether there were new areas of drusen or areas of drusen that had disappeared.

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.10 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.\(^8\) 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.12 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.13 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.10 Factors influencing change in visual acuity were assessed using multiple linear regression.

INCIDENCE OF CNV

As of the cutoff for this report, CNV had developed in 16 (30%) of the 53 treated eyes with laser burn quantitation in the FES. Analyses using the Cox proportional hazards model to examine the role of baseline characteristics showed that patients with past or current cigarette smoking and patients with more of their macula covered predominantly by larger (>125 µm) drusen were at increased risk for development of CNV. The influence of the intensity measures from the second treatment session was evaluated through the use of time-dependent covariates in the Cox model. The intensity measures from the second treatment session showed little influence on the risk for CNV after accounting for the level of the intensity measure from the initial treatment.
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 1.21 (95% CI, 0.66-2.22; 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.

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 = .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, particularly in the interval approximately 12 to 15 months after treatment (Figure 3).

DRUSEN REDUCTION

Forty-five (85%) of the 53 patients underwent evaluation for reduction in drusen at the 6-month visit. Choroidal neovascularization had developed 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 at 50% or more at 6 months. The odds ratio for drusen reduction associated with a 1-logarithm unit increase in 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 1.29 (95% CI, 0.89-2.84; 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).

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.27,11 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.\textsuperscript{5-11} 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.\textsuperscript{21}

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,\textsuperscript{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 Tso\textsuperscript{22} 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.\textsuperscript{23-25} 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.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
\textbf{Intensity} & \textbf{Drusen} & \multicolumn{2}{|c|}{\textbf{Temporal From Fovea}} & \multicolumn{2}{|c|}{\textbf{Nasal From Fovea}} \\
& & \textbf{Reduction} & \textbf{<1500} & \textbf{1500-3000} & \textbf{<1500} & \textbf{1500-3000} \\
\hline
\textbf{Low} & Yes & 13 (72) & 10 (56) & 11 (61) & 3 (17) \\
& No & 5 (28) & 8 (44) & 7 (39) & 15 (83) \\
\hline
\textbf{High} & Yes & 17 (94) & 15 (83) & 13 (72) & 8 (44) \\
& No & 1 (6) & 3 (17) & 5 (28) & 10 (56) \\
\hline
\end{tabular}
\caption{Data are given as number (percentage) of patients.}
\end{table}
Limitations of this study include a small sample size, since only 59 patients were in the treatment group of the CNVPT FES. The number of patients limits the statistical significance of the results reported, although the data remain strongly suggestive. In addition, the CNVPT protocol, which was designed to maximize drusen reduction in high-risk AMD patients, required a second laser treatment to be placed after 6 months in eyes that did not have a significant drusen reduction. Although the additional laser treatment was accounted for in the statistical calculations, the extra treatment makes quantifying the subsequent risk for development of CNV more difficult.

Our method for laser burn quantitation analyzes laser-induced color change between pretreatment and posttreatment photographs. Accordingly, distinctions among those patients with burns not visible with ophthalmoscopy or photography could not be performed. Moreover, we acknowledge that color change is a surrogate end point for tissue effect. We do not measure directly the photons absorbed. Although we normalize radiometrically pretreatment and posttreatment images, differences in global and local pigmentation may limit strict comparability. However, as clinical experience confirms that increasing laser absorption correlates strongly (albeit nonlinearly) with retinal color change from barely gray to bright white, the derived measures reflect the intensity of the laser application.

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.

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

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