Local Diabetic Retinopathy Prediction by Multifocal ERG Delays over 3 Years

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PURPOSE. To derive and validate a model for use in predicting local retinal areas in which nonproliferative diabetic retinopathy (NPDR) lesions will develop over a 3-year period, by using primarily the implicit time (IT) of the multifocal electroretinogram (mfERG).

METHODS. Eighteen diabetic patients were examined at baseline and at three annual follow-ups. Ophthalmic examinations, including fundus photographs and mfERG testing, were performed at each visit. Thirty-five retinal zones were constructed from the 103-element stimulus array, and each zone was assigned the maximum IT z-score within it based on 30 age-similar control subjects. Logistic regression was used to investigate the development of retinopathy in relation to baseline mfERG IT delays and additional diabetic health variables. Receiver operating characteristic (ROC) curves were used to evaluate the models.

RESULTS. Retinopathy developed in 77 of the 1208 retinal zones, of which 25 had recurring retinopathy. Multivariate analyses yielded baseline mfERG IT, duration of diabetes, and blood glucose concentration as the most important predictors of recurring retinopathy. mfERG ITs were not predictive of transient retinopathy. ROC curves based on the multivariate model for the prediction of recurring retinopathy resulted in an area under the curve of 0.95, sensitivity of 88%, and specificity of 98%. Ten-fold cross-validation confirmed the high sensitivity and specificity of the model.

CONCLUSIONS. The development of recurring retinopathy over a 3-year period can be well predicted by using a multivariate model based on mfERG implicit time. Multifocal ERG delays are promising candidate measures for trials of novel therapeutics directed at preventing or slowing the progression of NPDR. (*Invest Ophthalmol Vis Sci.* 2008;49:1622–1628) DOI: 10.1167/iovs.07-1157

Diabetic retinopathy continues to be the leading cause of blindness in the United States among people 20 to 74 years of age, accounting for up to 24,000 new cases of blindness every year.[1] Laser photocoagulation, developed several decades ago, remains the standard of care in diabetic retinopathy. Although therapeutic advances have been made, particularly as an adjunct to laser photocoagulation, nonproliferative diabetic retinopathy (NPDR) remains untreatable, beyond managing systemic factors. Even when metabolic control is considered, however, a certain percentage of patients still progress to diabetic retinopathy.[2] Part of the problem in finding treatments for NPDR lies in the fact that surrogate outcomes to evaluate therapeutics in earlier stages of the disease are necessary and have yet to be identified.[3,4] The other difficulty is that potential trials of therapeutics may require prohibitive sample sizes and follow-up times to assess a disease with relatively low occurrence rates, as measured by conventionally used outcomes.[5] Thus, there is a need to identify patients at risk of development of NPDR that goes beyond the conventional measures.

The suggestion of neural dysfunction in diabetes, in addition to vascular dysfunction, goes back many decades.[6] The means of studying this neural dysfunction in humans has involved electrophysiology of the eye and visual psychophysics. Several excellent reviews note deficits in full-field electroretinogram (ERG) oscillatory potentials, foveal cone ERGs, 30-Hz flicker delays, nonphotic electro-oculogram responses, visual acuity, contrast sensitivity, color vision, motion thresholds, and other functional measures.[7-9]

In the past several years our group has been investigating the implicit time of the multifocal electroretinogram (mfERG) as a prospective predictor for the development of diabetic retinopathy. The mfERG has been used to assess retinal function in many retinal disorders, including diabetic retinopathy.[10-13] The implicit time measure of the mfERG is spatially associated with retinopathy,[14] correlates with retinopathy severity,[14] and is a predictor for the development of retinopathy over a 1-year period.[15] This has not been shown for the amplitude measure of the mfERG. The first prominent positive peak (P1) of the mfERG response that our group has investigated is the easiest to measure, and the implicit time measure of P1 has one tenth of the coefficient of variation of the amplitude measure.[16] It has also been shown that the mfERG timing abnormalities (delays) can be used in a quantitative model to predict areas of retinopathy development with good accuracy: sensitivity of 86% and specificity of 84%.[17]

This quantitative model was the first to make predictions of diabetic retinopathy lesions in discrete retinal areas. The study, by Han et al.,[17] involved only one follow-up visit and thus could not examine whether the lesions they predicted were either more transient or sustained in nature. More recently, a review by Bearse et al.[16] included new data that extended the study by Han et al.[17] for another year. Bearse et al. examined only cumulative retinopathy occurrence, however, and did not examine the time course of the lesions.[16]

A retinal area that shows retinopathy lesions over a longer period represents more significant pathophysiological alterations—namely, increased vascular permeability and hypoxia. Thus, investigating these areas may be more clinically important than those areas that show retinal lesions that are present only transiently. It is well known that the very earliest clinical signs of diabetic retinopathy wax and wane. One study found

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that over a 2-year period, 52% of microaneurysms show spontaneous resolution.[18]

We addressed two primary questions in this study. First, given that predictions are more difficult over longer intervals,[16,19,20] can we still predict diabetic retinopathy lesions with good accuracy over a longer follow-up period? Second, given that early diabetic retinopathy lesions come and go,[18,21] are mfERG implicit times equally predictive of diabetic retinopathy lesions that may be transient as it is of those that are persistent?

To answer both of these questions, we continued our original longitudinal study for two more years, which allowed us to examine mfERG measures in areas without retinopathy at baseline and then the development of transient or more sustained retinopathy over the next 3 years.

METHODS

Subjects

Twenty-two diabetic subjects completed the entire study, with eight having been lost to follow-up from the model-making group of the previous 1-year study[17] and two new subjects having been gained. Of these 22 subjects, 4 had one of four sets of photographs (2.3%) lost or deemed upgradeable, leaving 18 subjects for analysis. Both eyes of each subject were used in data analysis. At baseline, the spherical equivalent of refractive error for the subjects was -2.28 ± 2.95 D and the logMAR acuity was -0.02 ± 0.08. These subjects are further described in Table 1.

All diabetic subjects had the following sets of data collected at baseline examination: medical history, blood glucose level measurement, mfERG recordings, and a comprehensive ophthalmic evaluation that included dilated fundus examination and 50° stereoscopic fundus photographs (model TRC-50X fundus camera; Topcon, Tokyo, Japan). The 50° field was chosen because it encompasses the testing field of the mfERG. Photographs were taken in accord with the protocol used in the EURODIAB study, which has been validated against the ETDRS protocol.[22-24] Fundus photographs were graded by a retinal specialist (SB) who was masked to any patient data, including the results of the mfERG recording. Lesions identified in photographs were then mapped onto the mfERG stimulus array (Fig. 1). Subjects completed four annual visits in which all procedures were repeated. The mean time between annual follow-ups was 1.1 ± 0.28 years.

Thirty eyes of 30 normal subjects were tested and used to normalize the mfERG measures. All normal subjects were free of ocular and systemic disease and had 20/20 or better best corrected visual acuity. The average age was 44.5 ± 11.5 years. All subjects had refractive errors between -6 and $+3$ D of equivalent diopter sphere.

The nature of the study and any potential consequences of study participation were explained to the study subjects, and written informed consent was obtained from all subjects before data collection. Procedures complied with the tenets of the Declaration of Helsinki, and the University of California Committee for the Protection of Human Subjects approved the protocol.

mfERG Recording

mfERGs were recorded with a commercial system (VERIS 4.3; EDI, Redwood City, CA). Subjects' pupils were fully dilated with 1.0%

tropicamide and 2.5% phenylephrine. The cornea was anesthetized with 0.5% proparacaine before a Burian-Allen bipolar contact lens electrode (Hansen Ophthalmic, Solon City, IA) was placed on the tested eye. A ground electrode was clipped to the right ear lobe, and the contralateral eye was occluded.

The stimulus consisted of 103 scaled hexagonal elements that subtended $\sim45°$ of the retina (Fig 1A). Subjects fixated a small target in the center of the stimulus array during the 8-minute recording session. Each hexagonal element was temporally modulated between white (200 cd/m^2) and black (<2 cd/m^2), according to a $2^{15} - 1$ binary m-sequence.[25] Room lights were kept on throughout the study session, providing an ambient illumination approximately equal to the average luminance of the stimulus. Data acquisition occurred in 16 segments that were each approximately 30 seconds in duration. The quality of recordings and fixation stability were monitored in real time, and contaminated segments were discarded and repeated. The retinal signals were amplified 100,000 times and band-pass filtered 10 to 100 Hz.[26] The mfERGs were processed using one iteration of artifact rejection and spatial averaging with one sixth of the surrounding responses before exporting the signals for data analysis.

Data Analysis

The mfERGs were analyzed as has been reported in detail.[17] The first-order kernel local mfERG implicit times were measured by using the template-scaling method.[27] Waveform templates were constructed from the mean local waveforms of the normal subjects. Each template was then multiplicatively scaled in amplitude and time until the maximum achievable least squares fit to the subject's local response was obtained. The subject's implicit time for a particular response was then derived as the time from the focal flash onset to the first prominent positive peak (P1) of the response (Fig. 1B). The mean and SD of each local mfERG implicit time measure was calculated from the normative data, and these were used to calculate a z-score for each local mfERG implicit time obtained from the diabetic subjects.

The 103 mfERG hexagonal stimulus elements were grouped into 35 zones (Figs. 1C, 1D), as reported previously,[17] to be spatially conservative. The zones were arranged in an approximately symmetric manner across the test area. Using zones instead of individual stimulus elements allows for possible spatial mismatches that could occur when mapping retinal lesions identified in photographs onto the mfERG stimulus array. Each zone was assigned the maximum z-score of the two to three elements that it consisted of. In accord with previous studies,[10,14] we set an a priori rule that elements that had *statfits* greater than or equal to 0.75 were not allowed to determine the z-score for a zone. A *statfit* of zero would mean that a response perfectly matched the scaled response template, an unexpected result. Conversely, a *statfit* of 1.0 would mean the template fitted the response as well as a flat line, implying that the response was extremely noisy or even absent. A *statfit* of less than 0.75 has been shown to indicate a false alarm rate less than 3%.[27] The a priori rule for trace rejection was unnecessary, because 3708 retinal elements (36 eyes × 103 retinal areas) were analyzed via the template scaling method, and none of these had a *statfit* greater than or equal to 0.75. The mean ± SD was 0.24 ± 0.09, with a range from 0.06 to 0.63, indicating very good fits of the scaled templates to the local responses.

TABLE 1. Patient Demographics at Study Entry

Subjects (*n*)	Baseline Retinopathy (Y/N)	Gender (M/F)	Age ± SD (Range, years)	DM Type (Type/*n*)	DM Duration ± SD (Range, years)	Blood Glucose ± SD (Range, mg/dL)
18 (36 eyes)	Y:6 N:30	M:9 F:9	50.4 ± 9.4 (28.3–63.6)	1:5 2:13	6.9 ± 4.9 (1–18)	169 ± 65 (89–323)

DM, diabetes mellitus.

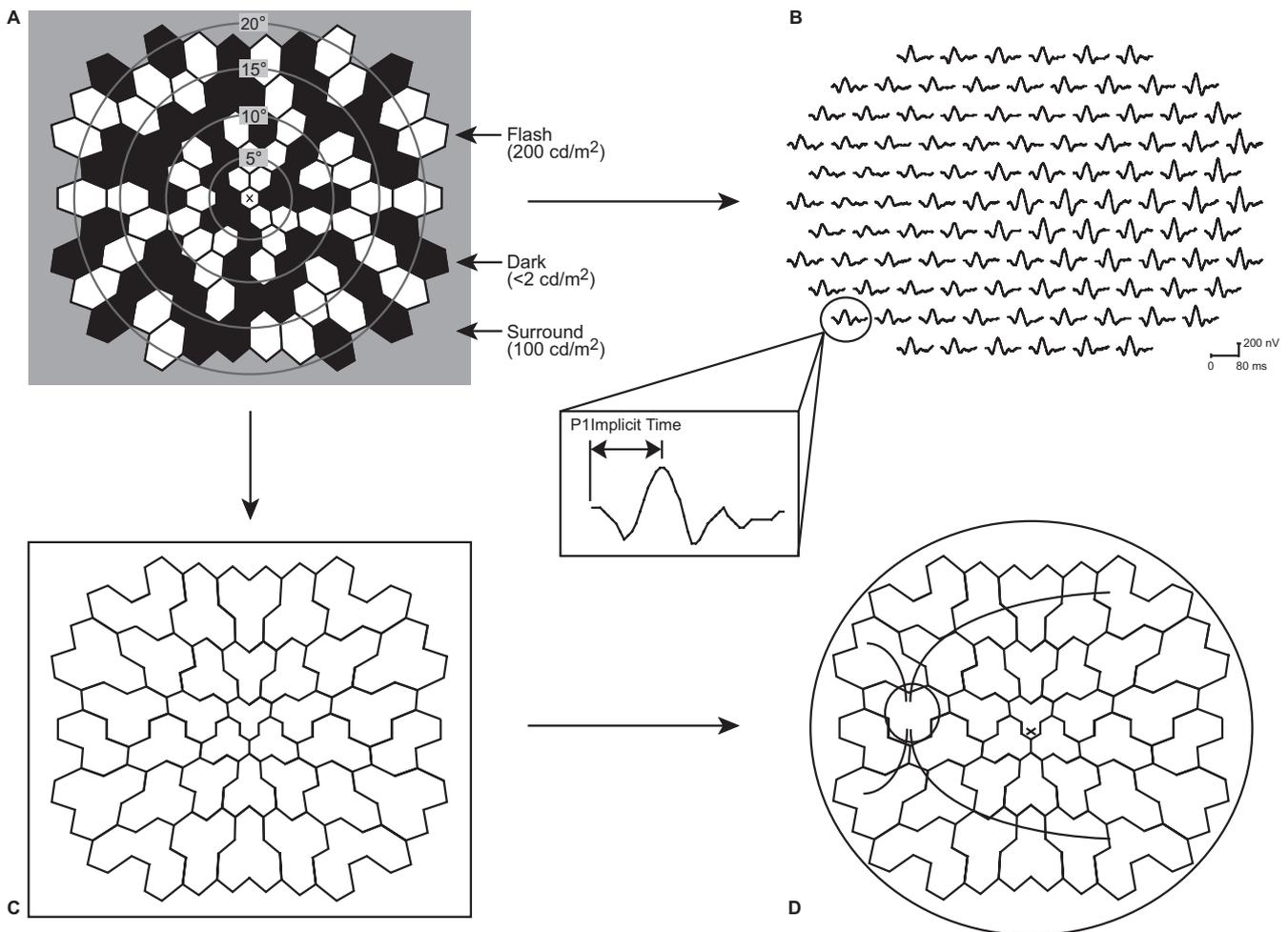**FIGURE 1.** (**A**) The stimulus configuration is an array of 103 hexagons scaled with retinal eccentricity. (**B**) A local response was derived for each of the 103 stimulus elements. Implicit time, as examined in this study, was measured from the onset of a focal flash to the first positive peak (P1). The mfERG traces are shown equally spaced for clarity. (**C**) Thirty-five retinal zones were constructed from the stimulus array. (**D**) The approximate spatial overlay of a schematic fundus photograph and the mfERG array of 35 retinal zones is shown.

Statistical Analysis

We investigated three dichotomized outcomes of retinopathy occurrence. A *cumulative* outcome (cumulative incidence) was defined as any zone with diabetic retinopathy at any time during the course of the three follow-ups. A *transient* outcome was defined as any zone with diabetic retinopathy that occurred at only one follow-up visit and was absent on the other two follow-up visits. Finally, a *recurring* outcome was defined as any zone having diabetic retinopathy that was present at two (not necessarily consecutive) follow-up visits or all three follow-ups. Retinal zones with baseline retinopathy were not included in the analysis, as we were interested in predicting the subsequent development of retinopathy in this study. All statistical analyses were performed in commercial software (Stata 9.2; StataCorp LP, College Station, TX). A criterion of $P < 0.05$ was used to define statistically significant results, except in the case of multiple *t*-tests and relative risks (RRs) where the conservative $P = 0.017$ (0.05/3 tests) was used.[28]

Logistic regression was performed to investigate the association between new retinopathy occurrence and mfERG implicit time, and several other risk factors: diabetic duration, blood glucose level, age, gender, diabetic type, and baseline retinopathy status. Because the sampling units (mfERG zones) may be spatially correlated within an eye, generalized estimating equations (GEEs) were applied to account for this possible correlation.[29,30] The GEE method allows the specification of a working correlation structure in the data. A compound symmetric covariance structure was chosen that assumes a common

covariance between mfERG zones within the eyes of a subject and independence between subjects. It is the ability to specify this structure that allows the method to estimate models that account for correlation. The correlation model is used to correct the variance-covariance matrix in a weighted regression. For example, highly correlated zones would have less influence on the final regression estimates. If the GEE method were not used, the covariance among the observations would not be accounted for and the standard errors of the regression coefficients would be artificially small. Robust standard errors were used with the GEE method to ensure appropriate inferences in the presence of any disparity between the empiric and specified covariance structures.

Since measures from the patients' two eyes might also be correlated, we assumed the same covariance structure between eyes as we did within eyes. The appropriateness of this structure, common with unordered data, was confirmed by the similarity between the naïve and robust variance estimators. Using the GEE method allows the formulation of an accurate model that accounts for covariance within and across eyes of subjects. Thus, analyses proceeded using both eyes of the subjects.

In building predictive models, we first performed univariate analyses of each risk factor as we have done previously.[16,17] We then built a preliminary multivariate model out of those risk factors that have been shown to be the most useful in the prior studies: mfERG implicit time, diabetic duration, and blood glucose level. Next, standard for-

ward stepwise regression was used based on the univariate analyses. The inclusion of an eye variable was used in the multivariate analyses to detect confounding, and none was found. For a risk factor to enter and remain in the model, criterion $P < 0.05$ and 0.10 were used, respectively.

Probabilities derived from logistic regression analyses were then used in receiver operating characteristic (ROC) curve analyses. Empiric ROC curves were derived from the known outcome of a zone and its associated probability derived from logistic regression. The area under the ROC curve (AUC) was used as a measure of discrimination and the optimal sensitivity and specificity were reported for each ROC curve.[31-33]

Ten-fold cross-validation was used both in model selection and validation.[34-36] In this technique, the data are randomly parsed into 10 data sets. Each set is used in validating models derived from the remaining nine sets. Generalized accuracy is then obtained from the average of the 10 validations.

RESULTS

Retinopathy Development

A total of 1260 retinal zones (36 eyes × 35 zones) were examined. Of these, 52 (4%) of the zones in six eyes had baseline retinopathy and were excluded from further analysis. The majority of retinopathy at baseline was microaneurysms, dot, or blot hemorrhages (69%), followed by hard exudates (17%) and areas of (nonfoveal) edema (14%). Over the course of the 3-year study period, new retinopathy developed in 77 retinal zones. Of these, 52 were classified as transient lesions and 25 were classified as recurring lesions. Transient lesions consisted mostly of microaneurysms, dot, or blot hemorrhages (79%) and areas of hard exudates (15%). One zone identified as having a cotton-wool spot and two zones as having edema were also transient. Recurring lesions in a zone were not necessarily singular or persistent. They are reported herein as the most severe type of lesion over the course of follow-up: 13 (52%) of the zones had microaneurysms, dot, or blot hemorrhages, 8 (32%) had hard exudates, and 2 each had cotton-wool spots and edema.

Relative Risks and Outcome Type

We first wanted to understand the general association between baseline mfERG implicit times and retinopathy development. Overall, retinal zones with abnormal implicit time z-scores (≥ 2) at baseline had a 71% increased risk of development of diabetic retinopathy over the 3 years (cumulative outcome, relative risk [RR] = 1.71, $P = 0.017$, 95% confidence interval [CI] = 1.10–2.68). Outcomes classified as transient retinopathy were not significantly associated with abnormal implicit time z-scores (RR = 0.75, $P = 0.41$, 95% CI = 0.38–1.48). Recurring retinopathy outcomes were significantly associated with abnormal baseline implicit time z-scores (RR = 7.6, $P < 0.001$, 95% CI = 3.21–18.03). These results suggest that retinal areas with recurring retinopathy underlie the marginally significant association found with the cumulative retinopathy outcome.

Baseline Implicit Times and Outcome Type

We then examined whether baseline mfERG implicit times were more abnormal in those retinal zones in which retinopathy developed than those in zones that remained free of retinopathy, and also whether the degree of abnormality varied by the type of outcome (cumulative, transient, or recurring). Table 2 shows that for outcomes classified as cumulative, there were marginal differences between the mean implicit time z-scores of zones in which retinopathy developed compared with zones in which it did not ($P = 0.04$). Zones that had retinopathy transiently did not differ statistically from zones in which it never developed ($P = 0.16$); however, there were significant abnormalities in zones in which recurring retinopathy developed ($P < 0.001$). Thus, recurring retinopathy is the only outcome that showed significant differences in baseline implicit times between areas in which retinopathy did and did not develop over 3 years.

Three-Year Locally Predictive Model Based on mfERG Implicit Time Alone

Given that recurring retinopathy lesions appear to underlie any significant associations between retinopathy development and abnormal implicit times at baseline and that these retinal lesions are more clinically significant, we investigated a model to predict recurring retinopathy using only the mfERG implicit time. Our logistic model yielded a significant and positive odds ratio (OR) for mfERG implicit time z-score (OR = 1.30, $P = 0.003$, 95% CI: 1.09–1.54), indicating that increases in mfERG implicit time are associated with an increased risk of development of retinopathy. In contrast, the mfERG implicit time was not significant in a model examining transient retinopathy (OR = 0.87, $P = 0.43$, 95% CI: 0.62–1.22). Therefore, this model for transient retinopathy was not developed further.

An ROC curve was plotted to assess the performance of the recurring model (Fig. 2A). Using the probabilities derived from the logistic regression model and the known retinopathy outcome (occurring or not), we plotted a function of sensitivity versus 1—specificity for various cutoff or criterion probabilities. The AUC ranges from 0 to 1.0 with 0.5 and 1.0 representing chance and perfect performance, respectively. ROC analysis of this model yielded an AUC of 0.83 with a sensitivity of 84% and specificity of 73% at a cutoff probability of 0.03.

Three-Year Locally Predictive Model Based on Multivariate Analysis

Previous models have been strengthened by the inclusion of other diabetic retinopathy risk factors.[16,17] The parameters for the multivariate logistic GEE model were baseline implicit

TABLE 2. Mean Implicit Times by Outcome Type and Retinopathy Development in Retinal Zones

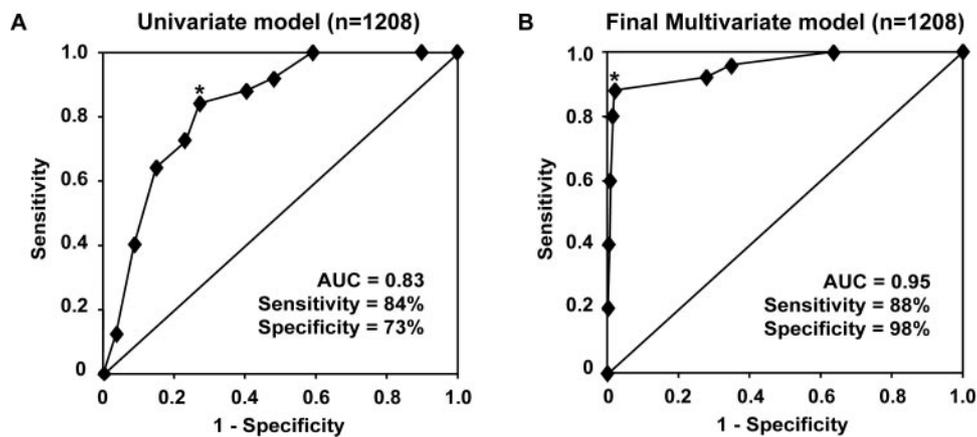| DR Outcome | DR Developed? | Sample Size | Baseline Implicit Time z-Scores | | P |
			Mean	SD	
Cumulative	Y	77	1.28	1.68	0.04
	N	1131	0.87	1.50	
Transient	Y	52	0.61	1.47	0.16
	N	1156	0.91	1.52	
Recurring	Y	25	2.67	1.16	<0.001
	N	1183	0.86	1.50	

FIGURE 2.   ROC curves for a univariate model of mfERG implicit time alone (**A**) and the final multivariate model (**B**) that predict recurring retinopathy over the course of 3 years in a local retinal area. Curves were generated from the prospective results of retinopathy outcomes and the derived probabilities from logistic regression by calculating the sensitivity and specificity for a range of criterion probabilities. *Diagonal line*: chance performance. The AUC provides an overall measure of discrimination. *Noted sensitivities and specificities. The multivariate model (which includes mfERG implicit time, diabetes duration, and blood glucose) performed significantly better than the univariate model based on the mfERG implicit time alone ($P < 0.001$).

time, diabetic duration, blood glucose level, and diabetic type (Table 3). Examination of this full model revealed that diabetic type, while significantly associated with development of retinopathy in this study, also had a large CI. This lack of precision in the estimate for diabetic type is not ideal in a predictive model, and thus we also derived a reduced model that did not include diabetes type (Table 3).

The ROC curves derived from the full and reduced (Fig. 2B) models had AUCs of 0.93 (95% CI = 0.92–0.95) and 0.95 (95% CI = 0.94–0.96), respectively, and these were not significantly different ($P = 0.07$) though they were both significantly superior to the univariate model ($P < 0.001$). The full and reduced models had sensitivities of 88% and specificities of 98% at cutoff probabilities of 0.03 and 0.12, respectively. In both multivariate models, diabetic duration and blood glucose level at the time of testing were, perhaps as expected, also significantly and positively associated with the development of retinopathy over a 3-year period (Table 3).

Ten-fold Cross-Validation of Multivariate Models

We cross-validated both the full and reduced multivariate models as a way of model selection and, more important, to obtain an estimate of the generalized accuracy of the models. Cross-validation gave 78% (SEM = 11%) sensitivity and 98% (SEM = 0.4%) specificity for the full model and 86% (SEM = 6%) sensitivity and 98% (SEM = 0.4%) specificity for the reduced model at cutoff probabilities of approximately 0.04 and 0.12, respectively. Thus, the reduced model performed as well as the

full model, and because it is more parsimonious, it is the preferred predictive model.

DISCUSSION

We have derived and validated a model to predict the development of recurring diabetic retinopathy lesions with exceptional accuracy in specific retinal locations over a 3-year period. The development of a model for recurring retinopathy was driven by the fact that abnormal mfERG implicit times were shown to be associated with an almost eight times greater risk of development of recurring retinopathy over 3 years, as opposed to transient retinopathy, which did not show an association. The model determines the probability of new retinopathy lesions based on mfERG implicit time, duration of diabetes, and blood glucose level.

ROC analysis showed that, with a cutoff probability of 0.12, the model has high accuracy (88% sensitivity, 98% specificity) in discriminating between those retinal areas that remain free of retinopathy (or have only transient retinopathy) and those areas in which recurring retinopathy develops. The model was validated with 10-fold cross-validation, which showed that it had accuracy (86% sensitivity, 98% specificity) extremely close to that empirically derived by ROC analysis. Cross-validation showed that the inclusion of diabetes type was unnecessary in the final model, and that if it were included, it would result in a lower sensitivity.

The predictive risk factors in this study are shared with our previous models,[16,17] with the exception of baseline retinop-

TABLE 3.   Parameters of the Full and Reduced (Final) Multivariate Models for the Development of Recurring Retinopathy

	Full Model			**Reduced (Final) Model**		
Variable (units)	**Coefficient**	**P**	**OR (95% CI)**	**Coefficient**	**P**	**OR (95% CI)**
mfERG IT (*z*-score)	0.50	0.002	1.65 (1.20–2.25)	0.58	0.006	1.78 (1.18–2.70)
DM duration (y)	0.32	0.001	1.38 (1.14–1.67)	0.46	0.001	1.59 (1.22–2.08)
Blood glucose (mg/dL)	0.02	<0.001	1.02 (1.01–1.03)	0.03	0.001	1.03 (1.01–1.05)
DM type* (0 or 1)	1.57	<0.001	4.79 (2.22–10.35)			

* Diabetes (DM) type was dichotomized to type 2 defined as 0 (absent) and type 1 defined as 1 (present).

athy status. Hyperglycemia has been well established as a risk factor for diabetic retinopathy and progression in large-scale, randomized, controlled clinical trials.[37,38] Similarly, duration of disease has been established as a prominent risk factor for retinopathy development in a large-scale epidemiologic study.[39,40] In the original study by Han et al.[17] the presence of any baseline retinopathy within an eye was a predictive factor, with a large OR, but also a wide CI. This result was because 11 of 12 diabetics in whom new retinopathy lesions developed 1 year later had some retinopathy at baseline, and retinopathy developed in only 1 of 16 who did not have any retinopathy at baseline. In this study, 4 of 6 eyes in which new retinopathy lesions developed and recurred over 3 years had retinopathy at baseline, and 1 of 30 eyes without baseline retinopathy had recurring retinopathy. Thus, baseline retinopathy status was not significant in the 3-year model, because 25% fewer eyes with baseline retinopathy had the recurring outcome measure than in the study by Han et al.

Han et al.[17] evaluated their multivariate model by applying it to data obtained from a second-year follow-up in the contralateral eye of eight subjects who were originally in the model-making group and to four new subjects who were not used in the derivation of the model.[17] It is unknown whether formal cross-validation of the data on the model would have significantly affected the final model reported by Han et al.

It is important to note that the implicit time of the mfERG provides the only locally variable parameter in the multivariate model and, therefore it alone allows the model to predict development of retinopathy in a specific retinal area. Knowledge of a retinal area at high risk of retinopathy also makes it possible, of course, to identify specific eyes and patients at high risk.

The mfERG P1 component analyzed in this study is generated primarily by bipolar cells,[41,42] which lie within the inner nuclear layer of the retina. Thus, the neurons primarily involved in generating the index of retinal function that we are investigating lie in the same intraretinal location as the vascular cells that are implicated in diabetic retinopathy lesions. Several recent reports have identified definite neuroglial dysfunction in diabetic mice. At the cellular level, there are neural alterations in the absence of morphologic changes in the vasculature.[43–46] In the first study to quantify neural cell loss in diabetes, Barber et al.[45] found that the inner nuclear layer, where the primary generators of the mfERG P1 implicit time lie, had the greatest reduction in thickness. Thus, these findings in mice complement the electrophysiology studies of human diabetic subjects.

A potential utilization of mfERG implicit time in randomized clinical trials would be as part of the inclusion criteria. In general, clinical trials enroll a large number of patients over a 3- to 5-year period. Often, the incidence of the primary or secondary outcome measures are low, and it is critical that they enroll patients at high risk for progressing toward or reaching those end points.[47] The mfERG implicit time has been shown to be predictive of new retinopathy lesions over a short period. Thus, its inclusion as entry criteria into a clinical trial would identify patients at much greater risk for development of diabetic retinopathy lesions. Enrolling these patients would ensure that evaluation of a novel therapeutic for preventing or slowing the progression of diabetic retinopathy would be more efficacious.

The development of surrogate outcomes also remains an important and critical task, as the current end points are of limited value in therapeutics targeted for earlier stages of diabetic retinopathy.[3,4] The implicit time of the mfERG has been shown to be a highly reproducible measure[48–50] that is correlated to diabetic retinopathy lesions[14,51] and predictive of development of retinopathy.[16,17] These are critical requirements for a surrogate outcome measure and in fact they satisfy three of the four criteria set forth by Berger.[51] The fourth criterion, evidence of modulation in the candidate measure by therapeutic intervention, can only be evaluated once the mfERG implicit time is used in a clinical trial, though it has been proposed that complete validation of a surrogate endpoint in a diabetic retinopathy trial should not be a prerequisite for drug approval.[3]

Models of the type we have formulated could be extended to predict specific lesion types—in particular, diabetic macular edema—and also to predict progression of retinopathy. We have already begun these longitudinal studies. Another step in evaluating the utility of mfERG implicit times in diabetic retinopathy is to know how and whether they change in eyes with diabetes, in eyes with diabetic retinopathy, in areas with retinopathy, and in areas without retinopathy. These questions are currently under investigation and have been reported in preliminary form (Bearse MA et al. *IOVS* 2006;47:ARVO E-Abstract 4732).

In this 3-year longitudinal prospective study, we examined the predictive ability of the mfERG to identify sites of new diabetic retinopathy. The study complements the most recent advances made in experimental diabetes that suggest that neuroglial dysfunction occurs before the overt development of morphologic changes in the vasculature. Further, it extends our previous work showing predictive ability of the technique over 1- and 2-year periods by examining retinal areas with recurrent retinopathic lesions. These lesions, which are indicative of more severe retinopathy and thus are of greater clinical interest, were associated with greater neural abnormalities at baseline than were lesions that were transient. The mfERG may be of value when used either as inclusion criteria to enroll, or as a surrogate outcome measure to be evaluated, in clinical trials of novel therapeutics for earlier stages of diabetic retinopathy.

Acknowledgments

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