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Validation of a Photographic Vitreous Haze Grading Technique for Clinical Trials in Uveitis

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Abstract

PURPOSE—To validate a photographic vitreous haze grading technique using a 9-step logarithmic scale in patients enrolled in a randomized, controlled clinical trial in uveitis.

DESIGN—Retrospective study of clinical trials methodology.

SETTING: University-based department of ophthalmology.

STUDY POPULATION: Baseline fundus photographs of patients with intermediate uveitis, posterior uveitis, or panuveitis enrolled in the Multicenter Uveitis Steroid Treatment (MUST) trial.

OBSERVATIONAL PROCEDURE: Grading of vitreous haze using a previously described photographic scale. Regrading of a subset of photographs to assess intraobserver agreement.

MAIN OUTCOME MEASURES: Interobserver and intraobserver intraclass correlation for photographic haze grading, and correlation between photographic and clinical vitreous haze scores, assessment of the clinical findings that significantly affect the photographic haze score.

RESULTS—Vitreous haze was graded in 271 eyes (142 patients) by 3 postgraduate ophthalmologists. The interobserver and intraobserver intraclass correlations were excellent, with correlation coefficients between 0.84 and 0.93. There was moderately strong correlation between the photographic and clinical vitreous haze scores ($r = 0.51$; $P < .001$), with significant differences among the mean and median photographic haze scores for the 3 lowest clinical grades of haze, 0, 1+, and 2+. Other parameters that correlated with photographic vitreous haze score included visual acuity of 20/50 or worse ($P = .003$), degrees of posterior synechiae ($P < .001$), lens abnormality ($P = .023$) or posterior capsule obscuration ($P = .001$), and amount of anterior vitreous cell ($P = .002$).

CONCLUSIONS—Photographic grading of vitreous haze with a 9-step logarithmic scale is a highly reproducible methodology that may be adaptable to use in future clinical trials.

Vitreous haze in patients with uveitis results from the accumulation of inflammatory cells and proteins in the vitreous.¹ In clinical trials, the amount of haze is used as a surrogate marker for the degree of inflammation, although this has not been validated. The

Nussenblatt scale for vitreous haze arranged representative 35-mm color fundus photographs of the disc–macula complex into a 6-step ordinal scale from 0 (clear vitreous) to 4 (no view of any fundus landmark) with an intermediate grade of trace.² The Standardization of Uveitis Nomenclature Working Group accepted the Nussenblatt scale, but recommended that trace haze be recorded as 0.5+ to permit mathematical calculations.³

Clinical grading of vitreous haze with the Nussenblatt scale is done with an indirect ophthalmoscope and 20-diopter lens by visually comparing the degree of haze on examination to a print of the scale. Limitations of the method include subjectivity, nonlinearity, and poor discrimination among the lower levels of haze that are present in most uveitis patients. A validation study comparing clinical haze gradings between 2 independent, skilled observers showed moderate levels of exact agreement (κ , 0.53) and excellent agreement within 1 grade (κ , 0.75) using the Nussenblatt scale.⁴

Recently, we demonstrated excellent interobserver and intraobserver agreement in grading vitreous haze using color fundus photographs and a 9-step, log-linear scale of vitreous haze; κ values averaged 0.91.⁵ Validation was performed with a library of 97 digitized clinical fundus photographs of patients with uveitis without other clinical information. The purpose of the current study was to compare vitreous haze scores obtained using the 9-step scale to assess fundus photographs taken of patients enrolled in a uveitis clinical trial on the same day that they were graded with the Nussenblatt scale and had other clinical data collected.

METHODS

STUDY POPULATION

The Multicenter Uveitis Steroid Treatment (MUST) trial ([Clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00132691) identifier NCT00132691) is a 23-site multicenter randomized clinical trial that assigned patients with active, noninfectious, sight-threatening intermediate, posterior, or panuveitis in a 1:1 ratio to 2 active comparator arms: intraocular implantation of a fluocinolone acetonide-containing drug delivery device (Retisert; Bausch & Lomb, Rochester, New York, USA) or oral corticosteroids with immunosuppressive agents as needed. The primary outcome measure was vision at 2 years. Secondary outcomes were retinal morphologic features; intraocular inflammation, including vitreous haze among other measures; mortality; cost effectiveness; and quality of life.⁶ Clinics were supplied a print of the Nussenblatt haze scale and were asked to use it to grade vitreous haze as part of each study eye examination. Vitreous haze was recorded as follows: 0, clear; 1+, opacities without obscuration of retinal details; 2+, few opacities resulting in mild blurring of posterior details; 3+, optic nerve head and retinal vessels significantly blurred but still visible; and 4+, dense opacity obscuring the optic nerve head. A grade of trace or 0.5+ vitreous haze was not recognized. Participants signed informed consent forms to participate in the MUST trial. The current study was approved as an ancillary study by the MUST trial executive committee.

STUDY PHOTOGRAPHS

Trained and certified photographers obtained baseline color photographs of 481 eyes of 255 MUST trial participants, and trained and certified ophthalmologists (J.L.D.) collected

baseline clinical data, including clinical grade of vitreous haze. The Wisconsin Photographic Reading Center excluded unreadable photographs, photographs obtained digitally rather than on film, and those not yet received at the Center by February 2009. Working with the Reading Center, one of us (B.M.) digitized the original color film slides with a Nikon Coolscan film scanner (Nikon, Inc, Melville, New York, USA) at 300 dpi and saved them in tagged image file format (TIFF). Two patients subsequently were excluded because there was no baseline data. Photographs of 271 eyes of 142 participants were included in this study (Table 1).

VITREOUS HAZE GRADE

Details of the 9-step photographic haze grading scale have been published previously.⁵ Three graduate ophthalmologists independently graded the MUST trial images in the same order in 3 batches to allow maximum attention and to lessen reader fatigue. The grading was performed in a quiet, dark, windowless room using a Pentium computer with monitor resolution of 1240 × 768 pixels (Dell Computer Corporation, Round Rock, Texas, USA). Windows toolbars were disabled to decrease reader distraction. The 9 standard scale images were displayed individually next to each study photograph as a mouse cursor was moved over corresponding numbered buttons on the computer screen. The reader matched the haze in the study photographs by sequentially displaying each standard image until the best match was obtained. The software automatically wrote the grading score into an internal database file and exported the data in Microsoft Excel (Microsoft, Redmond, Washington, USA) format.

STATISTICAL ANALYSIS

Grading reproducibility was assessed by calculating an interobserver intraclass correlation coefficient (ICC). A variance component analysis estimated variances between patient, between eyes nested within a patient, between readers, and residual variation. The ICC assessing reproducibility between readers included patient and eye variance components in the numerator and the sum of all variance components in the denominator. A second ICC was calculated to evaluate the correlation between the 2 eyes of patients, with between-patient variance in the numerator, and summed between-patient and between-eye-within-patient variance in the denominator. A subset of 40 photographs then was reevaluated 1 month after the initial grading to assess reader reproducibility over time. An intraobserver ICC thus was calculated for each reader.

The validity of the photographic haze grade was evaluated further by examining a subgroup of the 65 (49%) right eyes with clinical vitreous haze rank of 1+. They were categorized according to mean photographic vitreous haze scores from among 3 readers of 0 through 9. Confidence intervals (CIs) then were calculated overall and for each clinical site.

The contribution of different eye characteristics on photographic vitreous haze grading was examined with mixed models to account for the correlation between the 2 eyes of each patient. A forward stepwise procedure was performed manually to examine which eye characteristics were most important in a multivariate sense. Factors predictive of grader variability similarly were studied using mixed-model analysis, with the standard deviation

(SD) of photographic vitreous haze score as the dependent variable. Mixed-model analysis was also used to estimate the correlation between the average and SD of the haze score of both right and left eyes.⁷

RESULTS

READER GRADING REPRODUCIBILITY

The photographic vitreous haze grading technique demonstrated excellent agreement in scores among the 3 readers, with an interobserver ICC of 0.87 (values of more than 0.75 are considered good to excellent).⁸ There were substantial differences between the 2 eyes of patients with intraclass correlation between patients of 0.45. When a subset of 40 patient photographs was regraded independently by the 3 readers, there was excellent reproducibility, with an intraobserver ICC ranging between 0.84 and 0.93 among the 3 readers.

SDs among the 3 readers ranged from 0 to 1.7 for both right and left eyes. The correlation between the SDs of the mean photographic haze scores was calculated to evaluate whether higher photographic vitreous haze scores were associated with greater reader variability; an estimate of the correlation coefficient combining data from right and left eyes was -0.126 , which was not statistically significant ($P = .14$).

CORRELATION BETWEEN PHOTOGRAPHIC AND CLINICAL VITREOUS HAZE

Of the 271 eyes that underwent photographic vitreous haze grading, 265 (133 right eyes and 132 left eyes; 97.8%) had a documented clinical haze rank as part of the MUST trial protocol (Figures 1 and 2). There was moderately good correlation between the photographic vitreous haze grade and the clinical haze rank ($r = 0.51$; $P < .001$). As seen in Figures 3 and 4, the photographic haze grades mirror the distribution of the clinical ranks with finer gradations. For the 0, 1, and 2+ clinical grades of haze, the mean photographic scores (1.64 ± 1.23 , 2.80 ± 1.89 , 4.32 ± 2.13 , mean $\pm$ SD, respectively) were highly statistically different from each other ($P < .001$). Medians were similarly different (1.67, 2.33, 3.67, respectively; $P < .001$). Despite this, there was substantial overlap in the photographic haze scores of the 0, 1+, and 2+ clinical grades of haze. Seventy-five percent of photographs with a clinical grade of 0, 50% with a clinical grade of 1+, and approximately 15% with a clinical grade of 2+ had photographic scores of 2.33 or less. The clinical grade 1+ included photographic haze scores that also were represented in the upper 75% of clinical grade 0 and the lower 75% of clinical grade 2.

VALIDITY OF PHOTOGRAPHIC VITREOUS HAZE GRADING

Figure 5 shows the distribution of the haze scores among 65 right eyes with the modal clinical vitreous haze rank of 1+. Fifty-one (78%) of these 65 eyes had a photographic vitreous haze score of 1, 2, or 3, but all grades on the photographic scale were represented in the clinical haze rank of 1+. Mean photographic haze grades in this subgroup were analyzed on a per-clinic basis and CIs were calculated. Overall, the mean photographic vitreous haze grade for the 1+ group was 2.6 ± 1.9 (95% CI, 3.09 to 2.14). The width of the CI for each clinic varied from 1.3 to 55, but the intraclass correlation comparing within-clinic with

between-clinic variance was close to 0 and was not statistically significant. Representative photographs were selected from clinic 20 and clinic 6, which had small CIs indicating within-clinic consistency in grading. These photographs are displayed in Figure 6, where they are compared with 4 standard images from the photographic vitreous haze grader. Eyes clinically scored as having 1+ vitreous haze from clinic 20 had a mean photographic haze grade of 1.9 ± 1.19 (95% CI, 2.57 to 2.14). In contrast, 1+ haze eyes from clinic 6 had a mean photographic vitreous haze score of 5.08 ± 0.83 (95% CI, 6.41 to 3.75).

CONTRIBUTION OF EYE CHARACTERISTICS TO THE PHOTOGRAPHIC VITREOUS HAZE SCORE

Eye characteristics significantly associated with photographic vitreous haze scores included clinical grade of vitreous haze, anterior chamber flare, active uveitis, anterior chamber cells, vitreous cells, degrees of posterior synechiae, acuity worse than 20/50 resulting from uveitis (all $P < .001$), presence of corneal abnormalities or active panuveitis (both $P = .004$), active anterior uveitis ($P < .001$), active intermediate uveitis ($P = .029$), and a notation of obscured fundus ($P = .035$) or notation of obscured posterior chamber ($P = .057$) by the Reading Center. To evaluate the contribution of different eye examination findings on grading, variables were allowed inclusion into a model of grading in a forward stepwise fashion. The clinical vitreous haze rank was the first variable to enter the model ($r^2 = 0.28$). In order of statistical significance, clinical rank of vitreous haze ($P < .001$), degrees of posterior synechiae ($P < .001$), and posterior capsular opacification ($P = 0.001$) were included in a multivariate mixed model with P values less than .05. Table 2 provides slopes and CIs for all statistically significant variables. The influence of posterior synechiae and vitreous cells was quantifiable as an increase of 0.007 ± 0.002 haze grade per degree of synechiae (range, 0 to 360) and an increase in 0.50 ± 0.16 per rank of anterior vitreous cells (range, 0 to 4+).

EYE CHARACTERISTICS AFFECTING READER VARIABILITY

To evaluate the contribution of eye findings on the variability of photographic vitreous haze grading, eye characteristics were evaluated with a similar modeling strategy with the standard deviation of photographic vitreous haze score as the dependent variable. None of the variables evaluated was associated significantly with an increase in the SD of the haze gradings by the 3 readers.

DISCUSSION

The photographic vitreous haze grader was validated previously using a set of fundus photographs from miscellaneous patients in a uveitis clinic.⁵ Excellent interobserver and intraobserver reliability were found. Exact agreement and agreement within 1 grade were superior to that of clinical vitreous haze grading in 1 small validation study.⁶ Used to evaluate the MUST trial baseline photographic data set in this study, the photographic haze grader again was found to have excellent agreement between readers and excellent reproducibility within readers. Comparison of clinical haze ranks and photographic vitreous haze scores revealed a roughly linear relation between the two, demonstrating concurrent validity of the photographic scale. The photographic vitreous haze grader reliably can detect finer gradations of vitreous haze than the clinical scale. Discrepancies between clinical

grades of haze and photographic grades of haze may be attributable to the lower reproducibility of clinical grading or possibly to systematic differences between clinics.

Use of an actual clinical trials data set permitted investigation and quantification of other clinical parameters that may affect the photographic vitreous haze score. Although some discrepancies between the clinical vitreous haze rank and the photographic haze score may relate to imprecise grading by examiners or to systematic differences between clinical sites, others may derive from ocular characteristics that asymmetrically influence scores depending on whether clinical or photographic grading is being used. Our multivariate mixed model indicated that photographic vitreous haze grade was influenced principally by clinical vitreous haze rank, vitreous cell rank, and other measures of intraocular inflammation. We also calculated that 360 degrees of posterior synechiae may raise the photographic haze score by 2.5 and that posterior capsular opacification may raise it by 1.7. Lens and corneal opacification also had small, predictable effects. These analyses seem to confirm the predictive validity of the photographic vitreous haze score to reflect the true optical clarity of the eye, which usually is related to inflammation in a uveitic eye, but also demonstrate that eye characteristics that do not represent active inflammation can affect scoring in a quantifiable and predictable way. Conversely, eye characteristics that do represent active inflammation, such as peripheral vitreous haze, may not be captured adequately by this system.

The Nussenblatt haze scale was accepted as a means of clinically grading vitreous haze by the consensus group Standardization of Uveitis Nomenclature.³ In the MUST trial, vitreous haze assessed by the Nussenblatt scale was used as 1 indicator of intraocular inflammation along with anterior vitreous cells and a variety of other clinical measures. In other treatment trials (NCT00404612, NCT00685399, NCT01095250 at clinicaltrials.gov), 2+ clinical vitreous haze on the Nussenblatt scale has been used as an inclusion criterion, and a reduction of haze by 2 steps has been used as a principal outcome measure. The development of a precise and reproducible vitreous haze scale applicable to the large majority of patients with uveitis with relatively mild haze thus is important for clinical trials methodology.

In the MUST trial, the modal clinical vitreous haze score was 1+, occurring in approximately 50% of eyes. Approximately one third of eyes had 0 haze grade, approximately half of whom had inactive or never-present uveitis. Had the MUST trial required an inclusion criterion of clinical 2+ vitreous haze to permit the requisite reduction by 2 steps as an outcome measure, then only 15% of right eyes and 19% of left eyes could have been studied in the trial. Recalibration of the clinical 1+ vitreous haze group with photographic grading resulted in all but 22 of the 65 right eyes having a mean vitreous haze score of 2.0 or more, which would have permitted detection of an eventual 2-step change with treatment. Photographic grading therefore seems more sensitive than clinical grading of vitreous haze as well as more reproducible.

Limitations of the study include that it did not study variation in photographic technique that may affect grading, relying instead on certification of photographers and assessment of photographic quality by the Reading Center. The successful construction of a simulated

vitreous haze standard grading set is itself evidence that photographic artifact can simulate vitreous haze. Standardization of image resolution, brightness, contrast and color balance, selection of the best image, and calibration of monitors and lighting during grading likely would reduce bias related to the use of photographs for grading.

In conclusion, vitreous inflammation is assumed to be an important aspect of uveitis activity. The cellular component has been difficult to measure. Use of the composite measure vitreous haze to include both cells and protein has been hampered by the few gradations of the Nussenblatt scale, nonlinearity, and subjectivity. Photographic grading has the advantage of greater precision, log linearity based on optical blur, and the ability to train and test graders for reproducibility, but there are fundamental issues that remain unresolved. Additional study is needed to determine if the photographic vitreous haze scale can be used for haze grading in a clinical setting. It is unknown whether changes in the vitreous haze score over time will correlate with outcomes of known significance such as vision, so that haze can be considered a legitimate outcome measure for clinical trials. Further investigation into the usefulness of vitreous haze grading in uveitis is warranted.

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Biography

Brian Madow, MD, Clinical Assistant Professor, Department of Ophthalmology, University of South Florida received a medical degree in Bulgaria. He completed residency in Ophthalmology at the University of Pennsylvania, a fellowship in vitreoretinal diseases at the University of Tel Aviv and a medical retina fellowship at Bascom Palmer Eye Institute. He is certified by the American Board of Ophthalmology and a member of the American Academy of Ophthalmology and Association for Research in Vision and Ophthalmology.

FIGURE 1.

Bar graph showing the distribution of clinical vitreous haze ranks at baseline for 133 right eyes of 142 patients enrolled in the Multicenter Uveitis Steroid Treatment (MUST) trial. Eighty-four percent of right eyes were graded 0 or 1+, or 2 clinical grade steps.

FIGURE 2.

Bar graph showing the distribution of clinical vitreous haze grades at baseline for the 132 fellow left eyes of these patients. Eighty-one percent of left eyes were graded 0 or 1+, or 2 clinical grade steps.

FIGURE 3.

Bar graph showing the distribution of the photographic vitreous haze grade at baseline for 133 right eyes of 142 patients enrolled in the Multicenter Uveitis Steroid Treatment (MUST) trial. Eighty percent of right eyes were graded 0 to 5.0, a total of 6 photographic grade steps.

FIGURE 4.

Bar graph showing the distribution of the clinical vitreous haze grades at baseline for the 132 fellow left eyes of these patients. Seventy-nine percent of left eyes were graded 0 to 6.0, a total of 7 photographic grade steps.

FIGURE 5.

Bar graph showing the distribution of the mean photographic vitreous haze grades for the 65 right eyes with a clinical vitreous haze rank of 1+ on the Nussenblatt scale. The number of eyes with each score is shown on the vertical axis. The horizontal axis displays mean photographic vitreous haze scores of 3 readings clustered around whole numbers and including scores 0.33 higher and lower than each whole digit, except at the lowest and highest grades.

FIGURE 6.

Comparison of the photographic vitreous haze grader standards to Multicenter Uveitis Steroid Treatment (MUST) trial baseline photographs that were clinically graded as having 1+ vitreous haze rank on the Nussenblatt scale. The left column displays the photographic vitreous haze grader standards 1, 2, 3, and 4; grades 0, 5, 6, 7, and 8 are not shown.⁵ The 3 columns on the right contain MUST trial baseline photographs of right eyes that were assigned a clinical vitreous haze rank of 1+. Each row contains 3 photographs that had a mean photographic haze score in the range of the standard image in the first column. The first 3 rows contain 9 of the 15 vitreous haze rank 1+ right eyes from clinic 20 and demonstrate the ability of photographic grading to subdivide the clinical rank 1+ from that clinic into 3 separate grades. The bottom row contains 3 of 4 eyes that clinically were ranked as having 1+ vitreous haze by clinic 6. They are aligned with the photographic haze grader standard image 4, but in reality were graded as (Left) 4.0, (Middle) 5.0, and (Right) 6.0. The fourth right eye from this clinic (not shown) that was ranked 1+ clinically had a photographic haze score of 5.33, demonstrating a systematic difference in clinical haze ranking between clinic 20 and clinic 6.

TABLE 1

Baseline Clinical Characteristics of 271 Eyes of 142 Patients Enrolled in the Multicenter Uveitis Steroid Treatment (MUST) Trial

Characteristic	Right Eye	Left Eye
Mean IOP (standard deviation), mm Hg	14.0 (3.7)	14.0 (4.1)
Abnormal cornea, no. (%)	22 (16%)	23 (17%)
Posterior synechiae, no. (%)	24 (17%)	19 (13%)
Anterior chamber cells, no. (%)		
0	65 (48%)	71 (53%)
0.5	47 (35%)	38 (28%)
1	17 (13%)	14 (10%)
2	5 (4%)	5 (4%)
3	1 (1%)	6 (4%)
4	1 (1%)	1 (1%)
Anterior chamber flare, no. (%)		
0	69 (51%)	68 (48%)
1+	54 (40%)	50 (37%)
2+	12 (9%)	14 (10%)
3+	1 (1%)	3 (2%)
4+	0	0
Lens abnormalities, no. (%)	46 (34%)	45 (32%)
Posterior capsule obscuration, no. (%)	9 (6%)	6 (4%)
Vitreous haze, no. (%)		
0	48 (36%)	37 (28%)
1+	65 (49%)	70 (53%)
2+	14 (10%)	21 (16%)
3+	5 (4%)	4 (3%)
4+	1 (1%)	0
Vitreous cell, no. (%)		
0	29 (22%)	22 (17%)
0.5+	44 (33%)	32 (24%)
1+	38 (29%)	40 (30%)
2+	14 (11%)	29 (22%)
3+	6 (4%)	8 (6%)
4+	2 (1%)	2 (1%)
Fundus obscured	2 (2%)	1 (1%)
Active uveitis	93 (68%)	101 (75%)
Panuveitis	29 (21%)	30 (22%)
VA of 20/50 or worse	37 (27%)	48 (36%)

IOP = intraocular pressure; VA = visual acuity.

TABLE 2

Predicted Effect of Eye Characteristics on Photographic Vitreous Haze Score Expressed as a Coefficient of Variation with Standard Errors for Factors That Were Statistically Significant in a Multivariate Mixed Model

Clinical Characteristic	Coefficient $\pm$ SE	P Value
Clinical vitreous haze grading	0.76 ± 0.17	$< .001$
Posterior synechiae (0 to 360 degrees)	0.007 ± 0.002	$< .001$
	(per degree)	
Visual acuity 20/50	0.89 ± 0.29	.003
Posterior capsule obscuration	1.72 ± 0.51	.001
Vitreous cells (0 to 4+)	0.50 ± 0.16	.002
	(per grade)	
Corneal abnormality	1.1 ± 0.3	.002
Lens abnormality	0.6 ± 0.3	.023

SE = standard error.