Slitlamp Biomicroscopy and Photographic Image Analysis of Herpes Simplex Virus Stromal Keratitis

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Objective: To validate photographic biomaging for evaluating the severity of herpes simplex virus keratitis.

Methods: Stromal keratitis patients in the Herpetic Eye Disease Study was clinically measured with a slitbeam micrometer and then photographed at trial entry. Calibrated images of 169 eyes were analyzed for the size, location, and density of stromal keratitis and endotheliitis, with shape factor as a function of area and perimeter. Validity was assessed by comparing clinical and computerized measurements and by correlating the keratitis area with visual acuity. Logistic regression explored characteristics associated with larger or denser corneal inflammation.

Results: Stromal keratitis had a median area of 22.4 mm² (interquartile range, 12.8-31.6 mm²) with a median shape factor of 0.69 (interquartile range, 0.56-0.79); 126 eyes (75%) had their midpoint within 2 mm of the cornea’s geometric center. Photoanalytical area estimates of herpetic stromal keratitis correlated closely with clinical measurements (correlation coefficient, 0.83). Eyes with larger stromal keratitis had worse vision (correlation coefficient, 0.32) and were more likely to have iritis (P=.01). Necrotizing stromal keratitis was significantly whiter (P=.02).

Conclusions: Image analysis validly assesses the disciform geometry of herpetic stromal keratitis and confirms that increased severity is associated with uveitis and reduced vision.


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Group Information: The members of the Herpetic Eye Disease Study Group are listed on page 165.

The Herpetic Eye Disease Study is a set of multicenter, randomized, controlled clinical trials on the treatment and prevention of ocular infection and inflammation due to herpes simplex virus. In 2 trials, the effects of corticosteroid and antiviral agents were studied in patients with herpetic stromal keratitis and edema. Besides categorizing inflammatory severity, clinical examiners systematically measured and categorized the degree of stromal keratitis and obtained anterior segment photographs. We used these biomicroscopic measurements and calibrated photographs to examine the validity of digital image analysis in assessing corneal inflammation during herpetic eye disease. We also applied image morphometry to describe the geometric pattern of stromal keratitis and explored clinical features associated with severity.

METHODS

Participants

Patients with herpetic simplex virus stromal keratitis or endotheliitis who met eligibility criteria and gave informed consent were enrolled in 1 of 2 multicenter clinical trials at 9 clinical centers where the study had been approved by institutional review boards. One hundred six patients entered into a placebo-controlled trial of a topical corticosteroid (49 were assigned to placebo and 57 to prednisolone phosphate), and 104 corticosteroid-treated patients entered into a placebo-controlled trial of an oral antiviral agent (53 were assigned to placebo and 51 to acyclovir). All of the patients received trifluridine 1% solution.

Clinical assessment

Certified investigators performed corneal examinations at each study visit using a slitlamp biomicroscope (Haag-Streit BM 900; Haag-Streit USA, Inc, Mason, Ohio) fit with 10× eyepieces. With the transformer switched to 6 V, biomicroscopic examination began with the instrument’s illumination arm set upright with a fully opened filter and reticle. A fixation light in front of the contralateral eye helped to position the study eye so as to align the center of the region of stromal keratitis with the midpoint of the slitbeam. A sketch was made by outlining areas of stromal keratitis onto a printed diagram and adding reference lines to indicate length as the greatest diameter and width as its broadest perpendicular.

Beginning with a slitbeam positioned at 0° on the copivotal axis, the illumination housing was rotated to align a 10-unit-wide beam along the greatest length of stromal inflammation, and a continuous micrometer measured...
the longest diameter of stromal keratitis to the nearest 0.1 mm. For an axis longer than 8.0 mm, the eye was repositioned and the beam was adjusted to connect the center and border of stromal keratitis so that doubling this reading gave the lengthwise measurement. The illumination housing was then rotated 90° to measure the orthogonal dimension along the widest span of stromal keratitis. Length and width measurements were taken in triplicate and averaged for each region of nonnecrotizing or necrotizing stromal keratitis. Because a rectangular area overestimates an oval’s size, the total area of stromal keratitis used in the trial protocol was transformed to an elliptical area by applying a factor of π/4. The density of the inflammatory zone was categorized by aligning the illuminating slitbeam onto the center of the area of greatest density to distinguish whether the visibility of iris details was slightly blurred or was partially or completely obscured.

Best-corrected visual acuity was measured by a certified examiner who used subjective refraction to determine the optimal lens correction. Visual acuity of each eye was measured at 4 m with modified Bailey-Lovie charts retroilluminated at least 3 letters were correctly read. If visual acuity was 20/100 or worse, testing was repeated at 1 m after adding +0.75 sphere to the refraction. Snellen visual acuity was transformed to logMAR for analysis. For patients unable to discern letters at 1 m, the ability to count fingers, to detect hand motion, or to perceive light was evaluated and recorded as the logMAR equivalent of 2.0, 2.3, or 2.7, respectively.

PHOTODOCUMENTATION

Anterior segment photomicrography was performed with optical equipment (Zeiss Photo Slit Lamp; Carl Zeiss, Inc, New York, New York) consisting of a 35-mm single-lens reflex camera, 70/30 beam splitter, camera adapter with aperture diaphragm, 2× magnifier, and eyepiece graticule. The photographic protocol specified exposure parameters, image composition, and magnification settings. After attending a training seminar, photographers submitted acceptable slitlamp images from 2 patients to become certified.

Each session filmed a combined metric and contrast scale with a 1.5-mm black band, 2.2-mm white band, and matte gray background having 18% reflectance (Kodak, Rochester, New York). Ocular images were obtained at galilean settings of 6× and 10× to yield photographic magnifications of 1.4× and 2.2×, respectively, using dual diffuse illumination. Paired photographs were obtained with the eye gazing in primary position and then with the eye realigned to center the corneal inflammatory lesion within the image frame. Additional photographs were taken with a thin slitbeam, sclerotic scatter, and retroillumination.

Slide frames were labeled with the date, eye, protocol, and numeric code. Photographs were sent to a coordinating center that received slides at a median of 17 days after patient enrollment. Collected sets were subsequently forwarded to a reading center masked to treatment allocation where slides were checked for appropriate labeling, focus, composition, centration, and control of artifacts. If a center’s photographic sets were judged to be not acceptable or evaluable, corrective actions including photographer retraining, temporary center suspension of trial photography, and photographer recertification or replacement were taken. Photography was optional during the final 6 months of trial enrollment.

IMAGE PROCESSING

Diapositives archived in dark storage were digitized with a fixed-film scanning system (Super Coolscan 3000 ED; Nikon, Tokyo, Japan) having a charge-coupled linear sensor with optical resolution of 4000 pixels per inch. Images were obtained at a scan range of 3046×5782 pixels using neutralized correction and enhancement settings, imported to TIFs, and cached on a 1-TB
server (PowerEdge 4600; Dell, Round Rock, Texas). Image analysis software (SigmaScan Pro 5.0; Systat, Richmond, California) converted uncompressed images to linear gray-scale equivalents at a bit depth of 256 shade levels per pixel. Endpoints of the luminance scale were set to 0 and 255, and 18% gray was mapped to an intensity value of 128.

A pilot study exploring the feasibility of image analysis of biomicroscopic photographs captured transparencies with a mounted Vidicon camera (Dage-MTI, Michigan City, Indiana) and used a computerized image processor (1024XM; MegaVision, Inc, Santa Barbara, California). The interinstrument reproducibility of the SigmaScan and MegaVision systems for estimating the total area of stromal keratitis was examined in a subset of 52 patients photographed at study entry, using the Lin concordance correlation coefficient ($r_c$) with a 95% confidence interval (CI). Because results correlated ($r_c=0.71; 95\%\ CI, 0.57-0.84$), the SigmaScan system was used for analysis.

**MORPHOMETRY**

The width in pixels per millimeter was evaluated in triplicate for the mensural bands imaged at each photographic session. The luminance intensities at the upper and lower sections of the 18% gray background were recorded in triplicate and averaged, and similar readings were obtained from the white strip. Digitized clinical images were processed by boundary enhancement to accentuate the border of stromal infiltration and edema. Crispening was implemented through comparison of adjacent pixels, with the use of sharpening filters to enhance relative differences in neighboring pixel intensity values. Guided interactively by photographs taken with sclerotic scatter illumination, adjacent pixels were joined to outline a single area and a contrast histogram of pixels within the perimeter was plotted. Planimetric measurements and the averaged intensity of stromal keratitis were normalized to corresponding calibration scales.

An image analyst (B.M.M.) masked to clinical data recorded the area, perimeter, and axis dimensions. The maximal Feret diameter was the longest distance between parallel tangents. A shape factor estimating the relative circularity of the border of stromal keratitis was calculated as the product of $4\pi$ and the area divided by the perimeter squared. An overlay was modified to plot corneal disease and this template was centered on the midpoint of the corneal image by incircling the limbus (Figure 1). The geometric center of the area of stromal keratitis was mapped onto a composite chart by polar coordinates.

**STATISTICAL ANALYSIS**

Criterion validity was assessed by the Spearman rank correlation coefficient ($r_s$) with a 95% CI to compare the area of stromal keratitis estimated clinically with the area determined by computerized image analysis. Simple linear regression further compared the relationship between areas estimated by clinical and planimetric methods, and model robustness to observed data points was assessed with Bland-Altman plots and influence diagnostics using standardized delta-beta (dilbeta) analysis. Validity assessment compared the averaged intensity of stromal keratitis with the density as categorized clinically using the Kendall rank correlation coefficient ($\tau$) and Wilcoxon rank sum test. Face validity was examined by comparing initial area and intensity estimates with best-corrected visual acuity through the use of $r_c$. Internal consistency was examined by $r_c$ to com-
validity and consistency

Table. Relative Effects of Baseline Factors on Larger or Denser Stromal Keratitis Examined by Image Analysis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value (n=169)</th>
<th>Larger Stromal Keratitis*</th>
<th>Whiter Stromal Keratitisb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P Value</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>46 (18)</td>
<td>0.99 (0.83-1.17)</td>
<td>.87</td>
</tr>
<tr>
<td>Male, No. (%)</td>
<td>94 (56)</td>
<td>0.76 (0.41-1.42)</td>
<td>.39</td>
</tr>
<tr>
<td>Right eye, No. (%)</td>
<td>90 (53)</td>
<td>1.31 (0.70-2.42)</td>
<td>.40</td>
</tr>
<tr>
<td>Necrotizing keratitis, No. (%)</td>
<td>20 (12)</td>
<td>1.25 (0.47-3.32)</td>
<td>.65</td>
</tr>
<tr>
<td>Iritis, No. (%)</td>
<td>98 (58)</td>
<td>2.22 (1.18-4.18)</td>
<td>.01</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; OR, unadjusted odds ratio.

a Area greater than 20 mm².
b Intensity greater than 81 gray-value units.
c Age modeled as 10-year increments.

Of 210 patients enrolled in 1 of 2 clinical trials, 175 underwent slitlamp photography and 169 had 6× photographs of sufficient quality for analysis.

Results

Of 210 patients enrolled in 1 of 2 clinical trials, 175 underwent slitlamp photography and 169 had 6× photographs of sufficient quality for analysis.

Image Calibration

Compared with 220 pixels/mm for 6× magnification, 7 centers used photographic equipment that slightly exceeded expected calibration by an average of 6% to yield actual mean magnification ranging from 6.3× to 6.5× (Figure 1). One center was not significantly different from 6×, while another center’s photographic slitlamp camera resulted in average image magnification of 5.4×. Center values of pixel intensity for imaging pure white at the 6× setting ranged from a mean (SD) of 207 (11) units to 231 (5) units. For imaging the 18% gray background, values for each clinical center ranged from a mean (SD) of 79 (16) units to 157 (24) units (Figure 2), including 3 centers that did not significantly vary from a projected value of 128 gray-value units.

Validity and Consistency

The corneal inflammatory area estimated by photographic image analysis significantly correlated (P < .001) with the elliptical area estimated by clinical measurements (ρc=0.83; 95% CI, 0.78-0.88). However, 4 cases with clinically measured areas of 76, 96, 102, and 133 mm² and corresponding planimetric areas of 12, 30, 48, and 30 mm², respectively, exceeded 95% limits of agreement and showed unduly influential dbetas. After excluding these outliers for this comparison only, the slope of the regression line intersecting the origin (Figure 3) changed from 0.83 (95% CI, 0.78-0.89) to 0.98 (95% CI, 0.94-1.02). The clinical categorization of the density of stromal keratitis correlated (r=0.17; 95% CI, 0.10-0.25) with the averaged gray-scale intensity found by image analysis. The median averaged intensity value of the zone of stromal keratitis was 81 units (interquartile range [IQR], 59-103 units). The median intensity value of 64 units (IQR, 54-92 units) among 58 eyes (34%) clinically categorized as having mild haze was significantly lower (P < .001) than the median value of 95 units (IQR, 63-116 units) among 111 eyes (66%) judged to have moderate to marked opacification. Visual acuity correlated with the area of stromal keratitis (ρc=0.32; 95% CI, 0.17-0.45) and with its averaged gray-level intensity (ρc=0.35; 95% CI, 0.21-0.47). Comparison of 14 paired images in which one photograph was aligned with the eye’s visual axis and the other photograph had the area of stromal keratitis at its center showed that gaze position did not significantly alter estimates of area (ρc=0.99; 95% CI, 0.98-1.00) or intensity (ρc=0.93; 95% CI, 0.86-1.00).

Geometric Mapping

The median areas of stromal keratitis estimated by clinicians and by image analysis were 19.8 mm² (IQR, 10.2-29.2 mm²) and 22.4 mm² (IQR, 12.8-31.6 mm²), respectively. The mean (SD) dimension of the longest axis of the primary lesion was 5.1 (2.4) mm when measured clinically and 6.8 (2.1) mm when measured photographically, and the corresponding mean (SD) lengths of the orthogonal axis were 5.0 (2.8) mm and 5.0 (1.9) mm. The mean (SD) Feret diameter by image analysis was 5.4 (1.8) mm. The median perimeter of stromal keratitis was 21.5 mm (IQR, 16.5-26.0 mm). Stromal keratitis varied from a fusiform oval to near-circularity (Figure 4). The median shape factor was 0.69 (IQR, 0.56-0.79), and 40 eyes (24%) had a shape factor greater than 0.80. The geometric center of the zone of stromal keratitis was located at a median of 1.3 mm (IQR, 0.8-2.0 mm) from the corneal midpoint, and 126 eyes (75%) had their midpoint within the central 4-mm zone (Figure 5). Of 169 keratitis lesions among the 4 corneal quadrants, 71 (42%) were centered temporally, 48 (28%) inferiorly, 33 (20%) nasally, and 17 (10%) superiorly.

Attributes of Severity

Eyes with an area of stromal keratitis larger than 20 mm² had significantly increased odds of iritis (Table) by bio-

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microscopy (P = .03) and photogrammetry (P = .01). Necrotizing keratitis was more often associated with moderate to complete obscuration of the iris details (Table) on clinical examination (P = .03) and significantly exceeded the median intensity value of 81 gray-value units on densitometry (P = .02). Best-corrected visual acuity worse than 20/40 was found for 91 photographed eyes (54%). The odds of reduced vision occurred 3.08 (95% CI, 1.62-5.84) times as often as when the stromal keratitis area was larger than 20 mm² and 3.17 (95% CI, 1.69-5.95) times as often as when stromal keratitis intensity exceeded 81 gray-value units on digitized images. Eyes with stromal keratitis whose geometric center was located within 2 mm of the corneal center were 3.75 (95% CI, 1.78-7.89) times as likely to have visual acuity worse than 20/40 compared with eyes with more peripheral keratitis.

Ophthalmic examination and photographic imaging make ocular abnormalities visible.16 By capturing a pictorial record of eye disease, photography is an integral part of clinical practice and research. As grading systems of ocular photographs can be unreliable,17 computerized evaluation is emerging as an objective approach for quantifying the severity of ocular disorders.18

Biometric analysis has been successfully used to assess posterior lens capsule opacification, diabetic retinopathy, and age-related macular degeneration.14,19-21 Imaging technology also offers a way to evaluate conditions affecting the anterior segment such as conjunctivitis,22 corneal epithelial defects, and bacterial keratitis.23 Recent advances allow the size, shape, and haze of corneal disorders to be determined, nearly as accurately as with slitlamp biomicroscopy.9,24-26 Image analysis systems can make without redirecting the patient’s gaze.27

This cross-sectional study evaluated the performance of photographic image acquisition and processing in the initial assessment of herpes simplex virus keratitis. To assimilate clinical images taken on different days and at various centers, sessions included measurement and density calibration.27 Photographic slitlamp cameras varied slightly in magnification and lighting, so image standardization helped to compensate for technical variations in optics and luminance. Among the different adjustments in magnification, illumination, and angle of view, photographs taken at higher magnification or with a thin slitbeam proved less informative than those taken at a 6 × setting with diffuse illumination. Sclerotic scatter exaggerated the reflectance of stromal keratitis but highlighted the margins of corneal infiltration and edema and helped to guide planimetry. Shifting the eye position to bring the zone of stromal keratitis straight ahead gave results similar to those when having the eye in primary position, indicating that biomicroscopic measurements can be made without redirecting the patient’s gaze. Bioimaging revealed the geometry of herpes simplex virus stromal keratitis. With the use of an interactive edge-detection algorithm, the zone of corneal inflammatory opacification on digitized images was circumscribed by a curvilinear perimeter to yield a summed area of enclosed pixels. Mapping showed that stromal keratitis tended to cluster near the geometric center of the cornea, often in the temporal and inferior quadrants. Contour was evaluated by the shape factor, a geometric parameter that gauges the relative circularity of an object regardless of its border configuration. Despite an irregular perimeter for some eyes, half of the eyes in this series had stromal keratitis with a shape factor of 0.56 to 0.79, indicating an oval outline. Most could be morphometrically described as disciform. Larger keratitis became ronder and more likely to be associated with anterior uveitis. This study demonstrated that the size of herpes
simplex virus stromal keratitis estimated by image analysis correlated closely with elliptically transformed clinical area measurements.

The severity of stromal keratitis was also judged by its relative whiteness. Although a finer scale of clinical classification could improve precision and reliability,28-29 the majority of stromal keratitis was at least moderately hazy. After converting color images into gray tones, the averaged densitometric value of stromal keratitis provided a numerical measure of relative opacification29 and showed that densely white inflammation was a feature of necrotizing inflammation. Visual acuity worse than 20/40 occurred more often when stromal inflammation was larger, more opaque, or centrally located.

This validation study is subject to some technical limitations. We used scanned images of biomicroscopic photographs rather than direct digital imaging, although measurements should not depend on the image-capturing method.31 Image analysis was problematic if the margins of stromal inflammation and edema could not be clearly distinguished or if residual opacification remained from a prior episode. Light reflections from the tear film and variations of the iris surface also affected the ability to discriminate the transition zone at the border of stromal keratitis. Because clinical scales of ocular inflammation are not necessarily linear,32 comparing categorical disease severity with gray-level intensity was imprecise.

Further improvements in image resolution and microdensitometry offer prospects for using ophthalmic metadata in practice and teleresearch. Computerized analysis extends the clinical utility of anterior segment imaging from photodocumentation to quantitative description, comparison, and follow-up of corneal disease.

Submitted for Publication: April 7, 2008; final revision received September 2, 2008; accepted September 12, 2008.

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Financial Disclosure: None reported.

Funding/Support: This study was supported by the National Eye Institute through cooperative agreements EY07479, EY07480, EY07482, EY07483, EY07486, EY07487, EY07488, EY07489, and EY07496, by core grant EY02520 from the National Institutes of Health, and by Research to Prevent Blindness, Inc, and the Sid W. Richardson Foundation.

REFERENCES


