Corneal Epithelial Thickness Intrasubject Repeatability and its Relation With Visual Limitation in Keratoconus

ALFREDO VEGA-ESTRADA, MICHAEL MIMOUNI, ELIAS ESPLA, JORGE ALIÓ DEL BARRIO, AND JORGE L. ALIO

• PURPOSE: To evaluate intra-subject repeatability of anterior segment optical coherence tomography (AS-OCT) combined with placido disc MS-39 (CSO, Firenze, Italy) and correlate epithelial thickness measurements and the degree of visual limitation in keratoconus patients.

• DESIGN: Reliability analysis and cross-sectional study.

• METHODS: Setting: Vissum, Alicante, Spain. Subjects: Total of 167 eyes, of which 107 were from keratoconus patients and 60 were healthy. Procedures: Three repeated consecutive epithelial thickness measurements were taken: central 3.0 mm and the nasal, temporal, superior, and inferior regions at 3.0-6.0 mm and 6.0-8.0 mm. Main Outcome Measures: Intrasubject repeatability of epithelial indices was assessed and correlated to the degree of best-corrected visual acuity (BCVA).

• RESULTS: The intraclass correlation coefficient was ≥0.90, indicating highly repeatable measurements for both keratoconus and healthy eyes. In multivariate analysis, epithelial indices associated with worse BCVA were as follows: thinner 3 mm central ($P = .04$), thicker 8 mm superior ($P < .001$), and thinner 8 mm inferior ($P < .001$). Thickness central 3 mm decreased as keratoconus grading increased ($P = .002$). The superior-inferior ratio at 8 mm significantly increased as keratoconus grading increased ($P < .001$). In multivariate analysis thinner 3 mm central ($R^2 = 6.19\%$, $P = .04$), greater superior-inferior 8 mm ratio ($R^2 = 8.37\%$, $P = .004$), greater superior-inferior 6mm ratio ($R^2 = 2.67\%$, $P = .02$), and steeper K2 ($R^2 = 21.51\%$, $P < .001$) were associated with keratoconus with a combined area under the curve of 0.92.

• CONCLUSION: High repeatability of epithelial thickness measurements may be achieved in both keratoconus and healthy eyes with the MS-39. There is a significant correlation between epithelial thickness measurements and BCVA. These measurements may be of additional value in discriminating between keratoconus and healthy eyes. (Am J Ophthalmol 2019;200:255–262. © 2019 Elsevier Inc. All rights reserved.)

KERATOCONUS IS A BILATERAL, ASYMMETRIC, PROgressive ectatic condition that can significantly impair vision in affected individuals.[1] The reported prevalence of keratoconus ranges between 0.0003% and 2.3% depending on geographic location and criteria for diagnosis.[2] Several classification systems have been proposed to grade keratoconus, such as the Amsler-Krumeich,[3] keratoconus percentage index (KISA%),[4] Collaborative Longitudinal Evaluation of Keratoconus (CLEK),[5] ABCD,[6] and others.[7] Our research team has previously proposed and validated a grading system for keratoconus[8] and post-LASIK ectasia[9] based on visual limitation, namely best-corrected visual acuity (BCVA). The objective measurement of BCVA is readily available in day-to-day practice and is a continuous quantifiable measure that can be correlated with other continuous variables, allowing for more practical usage.[8,9]

Epithelial thinning at the corneal apex, together with epithelial thickening in the area around the apex (doughnut pattern), has been demonstrated by histopathology[10] and various imaging techniques to be representative features in keratoconic patients.[11,12] Reinstein and associates described the epithelial doughnut pattern using the Artemis very-high-frequency digital ultrasound arc-scanner (ArcScan Inc, Morrison, Colorado, USA)[12] and demonstrated that 3-dimensional thickness mapping of epithelial, stromal, and total corneal thickness profiles are useful in early diagnosis of keratoconus[11] as well as in identifying subclinical keratoconus.[13] Epithelial and stromal thickness maps measured with a noncontact anterior segment optical coherence tomography (AS-OCT) have been introduced, with several groups reporting parameters that allow to differentiate keratoconus from normal eyes and to classify the severity of the keratoconus.[14–16] In addition, it has been shown that the more advanced the keratoconus, the thinner the epithelium is at the central zone and the thicker the epithelium in the annular zone surrounding it.[11]

AJO.com Supplemental Material available at AJO.com.

Accepted for publication Jan 18, 2019.

From the Department of Cornea and Refractive Surgery, Vissum, Alicante, Spain (A.V.-E., E.E., J.A.B., J.L.A.); Keratoconus Unit of Vissum, Alicante, Spain (A.V.-E., J.A.B., J.L.A.); Division of Ophthalmology, Universidad Miguel Hernández, Alicante, Spain (A.V.-E., J.A.B., J.L.A.); Department of Ophthalmology, Rambam Health Care Campus, Haifa, Israel (M.M.); and The Ruth and Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel (M.M.).

Inquiries to Alfredo Vega-Estrada, Vissum Alicante, Calle Cabañal, 1 03016 Alicante, Spain; e-mails: alfredovega@vissum.com; rdioa@vissum.com

0002-9394/$36.00
https://doi.org/10.1016/j.ajo.2019.01.015

Recently, a novel standalone device that combines placido disc corneal topography and AS-OCT (MS-39; Costruzione Strumenti Oftalmici, Firenze, Italy) and provides automated measurements of the corneal epithelium was shown to have good repeatability in normal eyes and eyes following excimer laser surgery.[17] The potential advantages of this device are the ability to perform nontouch (as opposed to high-frequency digital ultrasound) automated measurements in order to perform a correct assessment of the corneal epithelium.

Therefore, the main purpose of the current study was to assess the correlation of MS-39 epithelial thickness measurements with BCVA in normal eyes and those with different stages of keratoconus. It also aimed to evaluate the repeatability of the measurements in patients suffering from keratoconus.

METHODS

• **PATIENTS:** This is a reliability analysis and cross-sectional study including consecutive eyes of keratoconus patients that presented for evaluation to Vissum, Alicante, Spain between May 1, 2017 and July 31, 2018. Keratoconus diagnosis was based in the following criteria: corneal topography revealing an asymmetric bowtie pattern with or without skewed axes or keratoconus sign on slit-lamp examination, such as localized stromal thinning, conical protrusion of the cornea at the apex, Fleischer ring, Vogt striae, or anterior stromal scar.[1] Additionally, a control group of patients with healthy cornea and corrected visual acuity of 0.1 in logMAR scale and none of the aforementioned ophthalmic or topographic alterations observed at the moment of examination in keratoconus subjects were included. Patients with previous ocular surgery or an active ocular disease other than keratoconus were excluded from the study. Ethical board committee approval from our institution was obtained for this investigation. In addition, informed consent to include clinical information in scientific studies was taken from all the participating patients, following the tenets of the Helsinki declaration.

• **MS-39 ANTERIOR SEGMENT OPTICAL COHERENCE TOMOGRAPHY AND PLACIDO DISK CORNEAL TOPOGRAPHY:** The MS-39 (software Phoenix v.3.6) is a standalone device that uses spectral-domain optical coherence tomography (SDOCT) and Placido-disk corneal topography to obtain measurements of the anterior segment of the eye and at the time of this study is marketed by Costruzione Strumenti Oftalmici (Firenze, Italy). After an autocalibration, the scanning process acquires (approximately in 1 s) 1 keratoscopy, 1 iris front image (used for the pupil detection), and a series of 25 SDOCT radial scans. The device uses a SLED light source at 845 nm, and provides an axial resolution of 3.6 μm (in tissue) and transversal resolution of 35 μm (in air). Each section measures 16 mm × 7.5 mm and includes 1024 A-scans. The ring edges are detected on the keratoscopy so that elevations, slope, and curvature data can be derived by the arc-step with conic curves algorithm. Profiles of the anterior cornea, posterior cornea, anterior lens, and iris are derived from the SDOCT scans. Data for the anterior surface from the Placido image and SDOCT scans are merged using a proprietary method. All other measurements for internal structures (posterior cornea, anterior lens, and iris) are derived solely from SDOCT data.

• **KERATOCONUS GRADING/SEVERITY:** Based on previous studies by our group, the keratoconus grading was based on spectacle BCVA.[8,18] Briefly, Group 1 consisted of subjects with a BCVA ≤ 0.05 logMAR; Group 2 consisted of patients with BCVA between 0.05 and 0.19 logMAR; Group 3 consisted of patients with BCVA between 0.19 and 0.40 logMAR; Group 4 consisted of patients with BCVA ≥ 0.40 logMAR.

• **MEASUREMENT PROTOCOL:** Subjects with soft or hard contact lenses were measured following a 1-week and 1-month period without contact lenses. All measurements performed with the MS-39 were performed according to the guidelines provided by the manufacturer and by an experienced technician (E.E.). Briefly, after adjusting the focus and achieving central alignment, the patient was asked to close and open his or her eyes and immediately afterward a measurement was performed. No drops were instilled in the eyes of the patient prior to measurement and adequate eyelid opening was verified. In addition, each measurement was assessed for sufficient quality in order to be included; otherwise, it was repeated. When the examination is correctly performed, the instrument displays a green checkmark acquisition quality, which means that the OCT image has an adequate coverage and the keratoscopy is correctly centered and covered. On the other hand, if the coverage of the OCT is not adequate or if the centration or coverage of the keratoscopy is not correct, the instrument will display an X sign in red, and in this case the technician repeats the examination until the acquisition quality is correctly displayed (Supplementary Figures 1 and 2; Supplementary Material available at AJO.com). This procedure was repeated 3 times consecutively so as to evaluate the repeatability of the MS-39. In order to avoid the effect of potential diurnal variations, measurements were performed in morning hours (9:00 AM and 2:00 PM).

• **KERATOMETRY MEASUREMENTS:** With the MS-39 instrument, the keratometry measurements are retrieved from the mean curvature of radius (mm) of the flat and steep anterior meridians of the cornea (the average of the 4-8 placido rings corresponding to 2.5-4.0 mm). Using the standard keratometric index (1.3375) the keratometric diopters are derived from the curvature (mm).

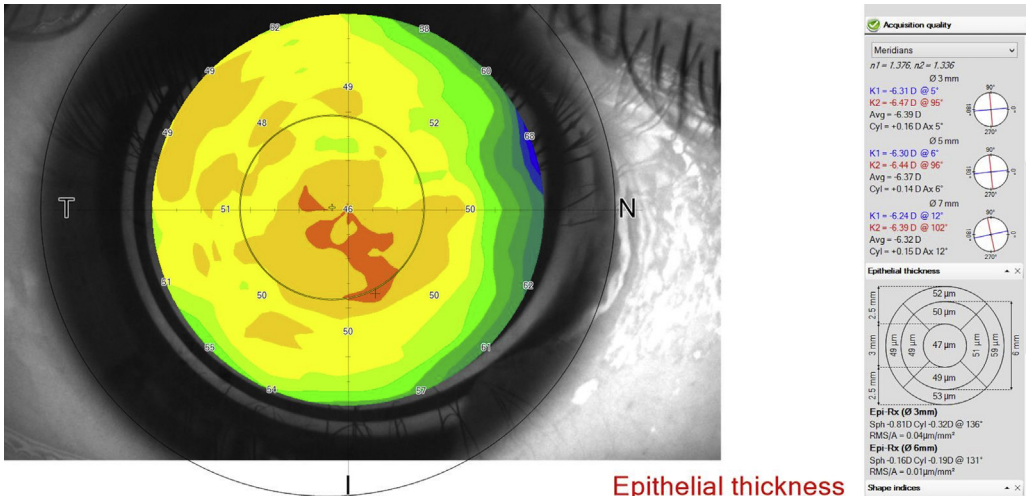

FIGURE 1. Appearance of the epithelial thickness map in a keratoconus patient captured by the MS-39; in the bottom right of the figure can be seen the different areas covered by the map—3.0 mm and the nasal, temporal, superior, and inferior regions at 3.0-6.0 mm and 6.0-8.0 mm—that were analyzed in the current study.

• **EPITHELIAL THICKNESS MEASUREMENTS AND CALCULATIONS:** The instrument calculates the epithelial thickness in the 8.0 mm zone and the built-in software automatically provides an epithelial thickness measurement map. For each image, sufficient quality was assessed and if it was insufficient (in terms of either signal strength or contained segmentation errors) the measurement was repeated. Nevertheless, the software is capable for manual editing in case of wrong edge detection. Supplementary Figure 3 (Supplementary Material available at AJO.com) provides an example of an OCT image and how the epithelial thickness is calculated. The present study evaluated the central reading over an area of 3.0 mm and the nasal, temporal, superior, and inferior regions at 3.0-6.0 mm and 6.0-8.0 mm (Figure 1).

The superior-inferior ratio was calculated by dividing the epithelial thickness at the superior region by the epithelial thickness at the inferior region.

• **REPEATABILITY:** Repeatability of epithelial thickness measurements was assessed using test-retest repeatability, coefficient of variance, and intraclass correlation coefficient. Test-retest was calculated by multiplying the pooled within-participant standard deviation by 2.77. The difference between 2 measurements for the same participant will be less than 2.77 within-participant for 95% of pairs of observations. Coefficient of variance (COV) was calculated as the within subject divided by the mean of the measurements (%). The intraclass correlation coefficient (ICC) was calculated using the 2-way mixed model and absolute agreement with an ICC $\geq$ 0.90 considered to be of high agreement.

• **SAMPLE SIZE ESTIMATION:** With repeatability of epithelial thickness measurements in healthy eyes as the outcome and based on a previous study,[17] in order to achieve a 15% confidence estimate at least 43 eyes would be required.[19] For the keratoconus group, given the lack of a previous study of epithelial thickness measurement repeatability in eyes with keratoconus, we aimed to achieve a higher degree of confidence (10% confidence estimate) and therefore at least 96 eyes would be required.[19]

• **STATISTICAL ANALYSIS:** Data were analyzed with the Minitab Software, version 17 (Minitab Inc, State College, Pennsylvania, USA) and MedCalc version 12.7.1.0 (MedCalc Software, Mariakerke, Belgium). Initially, normality of all data samples was evaluated by means of the Kolmogorov-Smirnov test. Spearman rank was used to analyze the relationship between BCVA and continuous variables. For the comparison of continuous variables between keratoconus and healthy eyes, Student *t* test was used for normally distributed variables and Kruskal-Wallis for nonparametric variables. A multivariate logistic regression analysis was performed in an attempt to predict BCVA based on epithelial thickness parameters. A multivariate binary logistic regression analysis was performed in an attempt to distinguish between keratoconus and healthy eyes based on epithelial thickness parameters with or without keratometry. Independent variables that reached a significance level of <0.10 in univariate analysis were included in multivariate analyses. Based on the binary logistic regression, the area under the receiver operating characteristic (ROC) curve (AUC) was determined to evaluate the discriminatory ability according to epithelial thickness parameters. The point with the larger Youden index, equal to sensitivity + specificity − 1, was defined as the optimal cutoff point. In all analyses a 2-sided *P* value $< .05$ was considered statistically significant. The main

TABLE 1. Repeatability of Epithelial Thickness in Keratoconus and Healthy Eyes

Parameter	Test-Retest Repeatability[a] (2.77*S_w)	Coefficient of Variance[b] (%)	Intraclass Correlation Coefficient[c]
Keratoconus (n = 107)			
K1	4.72	3.87	0.97 (0.96-0.98)
K2	3.93	3.00	0.98 (0.97-0.99)
Mean K	3.61	2.86	0.97 (0.96-0.98)
3 mm central	3.43	2.53	0.98 (0.97-0.99)
6 mm superior	3.98	2.77	0.97 (0.96-0.98)
6 mm inferior	4.69	3.30	0.97 (0.96-0.98)
6 mm temporal	4.49	3.16	0.98 (0.97-0.99)
6 mm nasal	3.20	2.16	0.98 (0.97-0.99)
8 mm superior	6.02	4.63	0.90 (0.85-0.93)
8 mm inferior	4.89	3.37	0.97 (0.96-0.98)
8 mm temporal	7.47	5.11	0.94 (0.92-0.96)
8 mm nasal	3.94	2.69	0.97 (0.95-0.98)
Healthy (n = 60)			
K1	0.46	0.38	0.99 (0.99-0.99)
K2	0.52	0.42	0.99 (0.99-0.99)
Mean K	0.47	0.39	0.99 (0.99-0.99)
3 mm central	5.62	3.86	0.99 (0.99-0.99)
6 mm superior	3.28	2.23	0.96 (0.94-0.98)
6 mm inferior	2.34	1.55	0.97 (0.95-0.98)
6 mm temporal	3.07	2.09	0.95 (0.93-0.97)
6 mm nasal	2.43	1.61	0.97 (0.95-0.98)
8 mm superior	7.54	5.37	0.93 (0.89-0.96)
8 mm inferior	2.75	1.79	0.97 (0.95-0.98)
8 mm temporal	3.58	2.41	0.94 (0.91-0.96)
8 mm nasal	4.16	2.65	0.93 (0.89-0.96)

[a]Test-retest was calculated by multiplying the pooled within-participant standard deviation by 2.77. The difference between 2 measurements for the same participant will be less than 2.77 within-participant for 95% of pairs of observations.[20]

[b]Coefficient of variance was calculated as the within subject divided by the mean of the measurements (%).

[c]Intraclass correlation coefficients (ICC) < 0.75 = poor agreement; ICC 0.75-0.90 = moderate agreement; ICC ≥ 0.90 = high agreement.[21]

outcome measures were correlation of MS-39 epithelial thickness measurements with BCVA in normal eyes and those with different stages of keratoconus, as well as repeatability of the epithelial thickness measurements in patients suffering from keratoconus.

RESULTS

THIS STUDY INCLUDED 167 EYES (N = 89), OF WHICH 107 WERE keratoconus eyes (n = 58) and 60 were normal eyes (n = 31). The mean age was 36.2 ± 14.1 and 52.8% were of male sex.

• **INTRASUBJECT REPEATABILITY:** The results of the repeatability testing of the MS-39 for keratoconus and healthy eyes are demonstrated in Table 1. For all examined parameters, the ICC was ≥ 0.90 indicating highly repeatable measurements for both keratoconus and healthy eyes. In the keratoconus group, the best repeatability measures (test-retest, COV, and ICC) were achieved at 6 mm nasal (3.20, 2.16%, and 0.98) and the worst (test-retest and COV) at 8 mm temporal (7.47 and 5.11%) and at 8 mm superior (ICC, 0.90). In the healthy group, the best repeatability measures (test-retest and COV) were achieved at 6 mm inferior (2.34 and 1.55%) and 3 mm central (ICC, 0.98) and the worst (test-retest, COV, and ICC) at 8 mm superior (7.54, 5.37%, and 0.93).

• **CORRELATION WITH BEST-CORRECTED VISUAL ACUITY:** All keratometry (K1, K2, and mean K) values positively correlated with BCVA (logMAR) ($P < .001$). Epithelial thickness parameters that significantly positively correlated with BCVA (logMAR) (worse visual acuity) were 6 mm superior-inferior ratio ($r = 0.28$, $P < .001$), 8 mm superior ($r = 0.22$, $P = .006$), 8 mm superior-inferior ratio ($r = 0.31$, $P < .001$), 8 mm temporal ($r = 0.16$, $P = .04$). Epithelial thickness parameters that significantly negatively correlated with BCVA (logMAR) (better visual acuity) were 3 mm central ($r = -0.29$, $P < .001$), 6 mm inferior ($r = -0.18$, $P = .02$), and 6 mm temporal ($r = -0.20$, $P = .01$).

• **MULTIVARIATE ANALYSIS OF EPITHELIAL MEASUREMENTS PREDICTING BEST-CORRECTED VISUAL ACUITY:** Multivariate analysis of epithelial thickness measurements predicting BCVA (logMAR) included all variables that were $P < .10$ in univariate analysis. Keratometric readings were omitted in order to emphasize the specific relationship between BCVA and epithelial thickness measurements. Factors that remained significant were 3 mm central ($T = -2.0$, $P = .04$), 8 mm superior ($T = 4.45$, $P < .001$), and 8 mm inferior ($T = -3.75$, $P < .001$). A main effect plot, based on the multivariate analysis, clearly demonstrates that an increase in 3 mm central and 8 mm inferior thickness was associated with a decrease in BCVA logMAR (better visual acuity) (Figure 2, Left and Middle), while an increase in 8 mm superior was associated with worse visual acuity (Figure 2, Right).

• **KERATOCONUS SEVERITY AND EPITHELIAL THICKNESS:** The thickness of the central 3 mm decreases as keratoconus grading increases (Figure 3, Left) ($P = .002$). The superior-inferior ratio at 8 mm significantly rises as keratoconus grading increases (Figure 3, Right) ($P < .001$).

• **EPITHELIAL THICKNESS IN KERATOCONUS VS HEALTHY EYES:** The keratoconus and healthy subjects were of similar age (36.4 ± 15.9 vs 35.8 ± 9.9 years, respectively, $P = .83$). Table 2 depicts a comparison between keratoconus and healthy eyes. The keratoconus eyes had higher keratometry

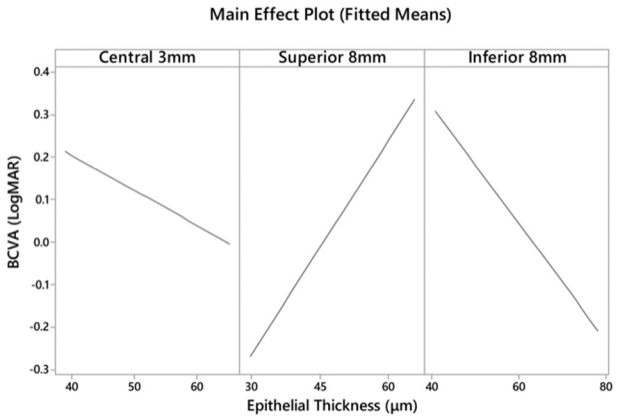

FIGURE 2. Main effects plot of the 3 parameters that were significant in multivariate analysis of BCVA (logMAR) where (Left) an increase in 3 mm central and (Right) 8 mm inferior thickness are associated with a decrease in BCVA logMAR (better visual acuity) while (Middle) an increase in 8 mm superior is associated with worse visual acuity.

values ($P < .001$) and thinner 3 mm central and 6 mm inferior thickness ($P < .001$ and $P = .04$, respectively). Keratoconus eyes had thicker 6 mm superior ($P = .03$), 8 mm superior ($P = .005$), and 8 mm temporal ($P = .002$) thickness.

• **MULTIVARIATE ANALYSIS OF KERATOCONUS VS HEALTHY EYES:** In multivariate analysis with keratoconus vs healthy eyes as the dependent factor and all variables that were $P < .10$ in univariate analysis as independent variables, 3 mm central ($R^2 = 6.19\%$, $P = .04$), superior-inferior 8 mm ratio ($R^2 = 8.37\%$, $P = .004$), superior-inferior 6 mm ratio ($R^2 = 2.67\%$, $P = .02$), 8 mm temporal ($R^2 = 7.93\%$, $P = .15$) and K2 ($R^2 = 21.51\%$, $P < .001$) achieved an R^2 of 46.66% (meaning that the model explained 46.66% of the variation in the outcome variable assessed, keratoconus vs healthy).

• **RECEIVER OPERATING CHARACTERISTIC CURVE ANALYSES OF DIFFERENTIATING KERATOCONUS FROM HEALTHY EYES:** The ROC curve analyses are depicted in Figure 4. Using the 3 mm central only as a discriminatory factor at an optimal cutoff of ≤50.3 µm, sensitivity was 57.9% (95% confidence interval [CI] 48.0%-67.4%), specificity 78.3% (95% CI 65.8%-87.9%), and AUC 0.68 (95% CI 0.60-0.65) (Figure 4, Top left). Using the 6 mm superior-inferior ratio only at an optimal cutoff of ≥0.99, sensitivity was 66.0% (95% CI 56.2%-75.0%), specificity 78.3% (95% CI 65.8%-87.9%), and AUC 0.71 (95% CI 0.64-0.78) (Figure 4, Top right). With a ROC curve based on the multivariate binary logistic regression analysis there was a sensitivity of 74.0% (95% CI 64.0%-82.4%), specificity of 96.7% (95% CI 88.5%-99.6%), and AUC 0.92 (95% CI 0.86-0.95) (Figure 4, Bottom left). A ROC curve based on multivariate binary logistic regression analysis after omitting the keratometry values achieved a sensitivity of 57.3% (46.8%-67.3%), specificity of 95.0% (86.1%-99.0%), and AUC of 0.81 (0.74-0.87) (Figure 4, Bottom right).

DISCUSSION

THIS STUDY DEMONSTRATED THE HIGH REPEATABILITY OF the MS-39 epithelial thickness measurements in both keratoconus and healthy eyes. In addition, a significant correlation was found between epithelial thickness measurements and the degree of visual impairment in keratoconus patients. Last, the discriminatory value (keratoconus vs healthy) of epithelial thickness measurements with and without keratometry was assessed and AUC values reported. To the best of our knowledge, this is the first study to report such data with the AS-OCT MS-39 instrument.

Assessing the repeatability of an instrument is a crucial step in its validation. Savini and associates[17] previously assessed the MS-39 in normal eyes and eyes following excimer laser treatment and reported high repeatability and agreement with a rotating Scheimpflug camera (Pentacam HR, Oculus Optikgerate GmbH, Firenze, Italy) and a rotating Scheimpflug camera combined with Placido disk topography (Sirius, Costruzione Strumenti Oftalmici). Similarly, in the current study, for all examined parameters the ICC was ≥0.90, indicating highly repeatable measurements for both keratoconus and healthy eyes. In addition, both studies reported a central epithelial thickness measurement of ~53 ± 3 µm in healthy eyes with an ICC of ~0.99, similar to those of previous reports of other AS-OCT devices and the Artemis,[22] supporting the findings of the current study.

Reinstein and associates described the masking effect of the highly regenerative corneal epithelium on irregularities of the stroma in corneal ectatic diseases.[11,12] In the current study, a thinner 3 mm central, thinner 8 mm inferior epithelial thickness, and thicker 8 mm superior epithelial thickness were associated with worse visual acuity in both univariate and multivariate analyses. Similarly, as keratoconus grading increased and visual acuity deteriorates, the 3 mm central thickness decreased and the superior-inferior ratio at 8 mm increased, with these same factors discriminating between keratoconus and healthy eyes in both univariate and multivariate analyses. These findings are supported by those of Temstet and associates, who reported, with AS-OCT, that as keratoconus severity increased the epithelial thickness became thinner and the superior inferior difference increased.[23] Similarly, Li and associates described thinner epithelial thickness and greater superior inferior difference in keratoconus eyes when compared to normal eyes.[24] It has been proposed that the epithelial thinning in the areas may compensate for the localized anterior protrusion of the cornea owing to stromal thinning in keratoconus.[12]

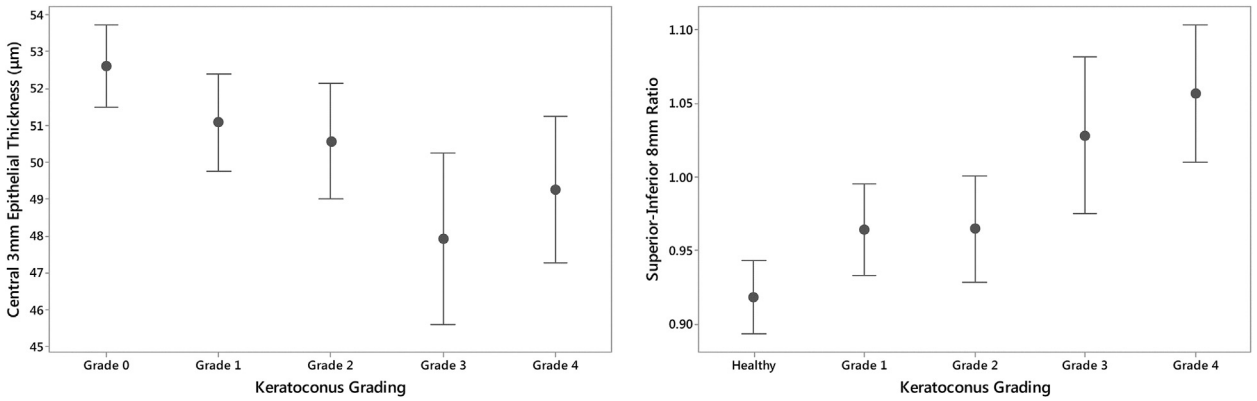

FIGURE 3. (Left) The thickness of the central 3 mm significantly decreases as keratoconus grading increases ($P = .002$). (Right) The superior-inferior ratio at 8 mm significantly rises as keratoconus grading increases ($P < .001$).

TABLE 2. Comparison of Keratoconus and Healthy Eyes

Parameter	Keratoconus (N = 107)	Healthy (N = 60)	*P* Value
K1 (D)	44.90 ± 3.98	43.42 ± 1.27	.001
K2 (D)	48.18 ± 4.61	44.16 ± 1.25	<.001
Mean K (D)	46.44 ± 4.13	43.79 ± 1.23	<.001
3 mm central (µm)	50.19 ± 5.14	52.61 ± 2.89	<.001
6 mm superior (µm)	54.52 ± 4.92	53.12 ± 3.45	.03
6 mm inferior (µm)	53.07 ± 5.85	54.46 ± 2.95	.04
6 mm temporal (µm)	52.79 ± 5.09	53.10 ± 2.91	.62
6 mm nasal (µm)	54.98 ± 4.70	54.38 ± 2.68	.29
8 mm superior (µm)	53.36 ± 4.53	50.97 ± 5.41	.005
8 mm inferior (µm)	54.22 ± 6.12	55.50 ± 3.25	.08
8 mm temporal (µm)	55.64 ± 5.03	53.66 ± 3.06	.002
8 mm nasal (µm)	56.92 ± 4.39	56.54 ± 3.60	.56

Imaging modalities and the potential of their indices to correctly discriminate between keratoconus and healthy eyes has been the subject of intense research.[25] Reinstein and associates reported epithelial thickness indices, measured with the Artemis device, and their association with keratoconus.[11,12] Since then, noncontact, high-axial-resolution OCTs have also enabled using epithelial thickness indices to discriminate between keratoconus and healthy eyes.[14,15,23] In the current study, no single epithelial thickness measurement yielded satisfactory sensitivity (57.9%-74.0%), specificity (78.3%), or AUC (0.68-0.71). The combination of epithelial indices improved the AUC to 0.81 and the addition of keratometry values further improved the AUC to 0.92. None of the AUC values are sufficiently reliable for the diagnosis of keratoconus. Pircher and associates recently reported that with ultra-high-resolution AS-OCT the AUC was higher with the ratio between the thinnest point and the diagonally opposing point (0.98) (a parameter not analyzed in the current study) than with the superior inferior ratio (0.83).[14] It therefore seems as though the built in indices provided by the generated thickness maps are insufficient and that automated calculation of additional epithelial thickness indices would be beneficial. In addition, a future combination of indices from several different anterior segment imaging modalities will probably lead to the highest sensitivity and specificity values.

In the current study a significant correlation between epithelial thickness and keratoconus severity as classified by the visual deterioration was observed. These findings are of a major relevance, as success and failure of a specific therapeutic in keratoconus patients like intracorneal ring segments is closely related to the degree of visual acuity limitation. Moreover, the relationship between epithelial thickness measurements and the different degrees of keratoconus may indirectly represent the evolution of the disease; thus, epithelial thickness could be used as an adjuvant variable to assess keratoconus progression. Nevertheless, progression of a disease is a condition that should be evaluated in longitudinal studies.

The main limitation of this investigation is that epithelial thickness indices previously reported to be associated with keratoconus, such as map standard deviation, pattern standard deviation, minimum maximum difference, diagonally opposing points, and others, were not evaluated.[14,24] In addition, these measurements and their location relative to the patients "cone," which may affect visual acuity, were not assessed. Instead, the current study focused on the parameters provided by the epithelial thickness maps, and therefore future studies may consider incorporating such measurements as well.

In conclusion, high repeatability of epithelial thickness measurements may be achieved in both keratoconus and healthy eyes using the AS-OCT MS-39 instrument, and there is a significant correlation between epithelial thickness measurements and the degree of visual impairment in keratoconus patients. The epithelial thickness of keratoconus eyes is thinner centrally, thicker superiorly, and thinner inferiorly when compared to normal eyes and a high superior-inferior epithelial thickness ratio is characteristic of keratoconus.

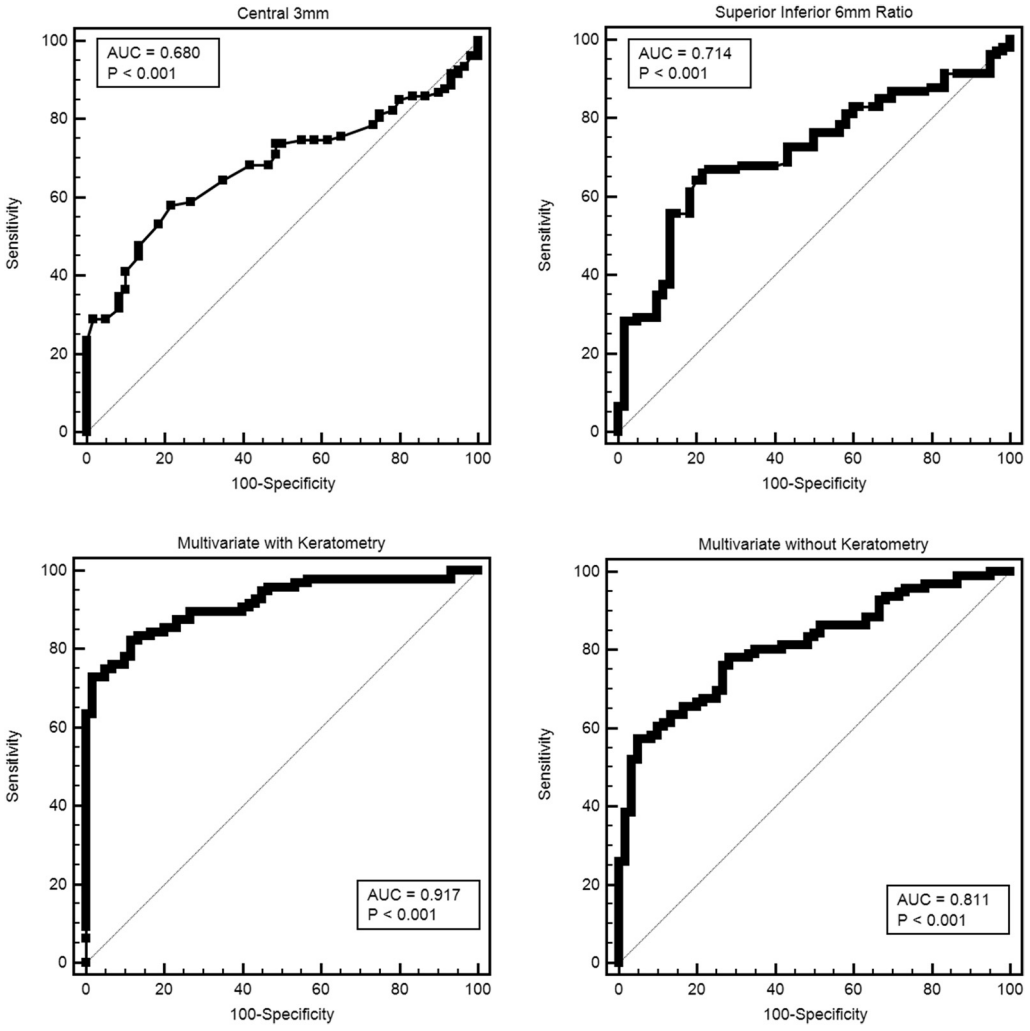

FIGURE 4. (Top left) Receiver operating characteristic curve analysis using the central 3 mm epithelial thickness only as a discriminatory factor at an optimal cutoff of ≤50.3 μm sensitivity was 57.9% (95% confidence interval [CI] 48.0%-67.4%), specificity 78.3% (95% 65.8%-87.9%) and area under the ROC curve (AUC) 0.68 (95% CI 0.60-0.65) ($P < .001$). (Top right) Using the 6 mm superior-inferior ratio only at an optimal cutoff of ≥0.99, sensitivity was 66.0% (95% CI 56.2%-75.0%), specificity 78.3% (95% 65.8%-87.9%), and AUC 0.71 (95% CI 0.64-0.78) ($P < .001$). (Bottom right) ROC curve based on the multivariate binary logistic regression analysis there was a sensitivity of 74.0% (95% CI 64.0-82.4%), specificity of 96.7% (88.5%-99.6%), and AUC 0.92 (95% CI 0.86-0.95) ($P < .001$). (Bottom left) ROC curve based on multivariate binary logistic regression analysis after omitting the keratometry values achieved a sensitivity of 57.3% (46.8%-67.3%), specificity of 95.0% (86.1%-99.0%), and AUC of 0.81 (0.74-0.87).

FUNDING/SUPPORT: THIS PUBLICATION HAS BEEN CARRIED OUT IN THE FRAMEWORK OF THE RED TEMÁTICA DE INVESTIGAción Cooperativa en Salud (RETICS), reference number RD16/0008/0012, financed by the Instituto Carlos III – General Subdirection of Networks and Cooperative Investigation Centers (R&D&I National Plan 2008-2011) and the European Regional Development Fund (Fondo Europeo de Desarrollo Regional FEDER). Financial Disclosures: Jorge L. Alió is consultant for Costruzione Strumenti Oftalmici (CSO). The following authors have no financial disclosures: Alfredo Vega-Estrada, Michael Mimouni, Elias Espla, and Jorge Alió del Barrio. All authors attest that they meet the current ICMJE criteria for authorship.

REFERENCES

1. Rabinowitz YS. Keratoconus. *Surv Ophthalmol* 1998;42(4):297–319.
2. Kennedy RH, Bourne WM, Dyer JA. A 48-year clinical and epidemiologic study of keratoconus. *Am J Ophthalmol* 1986;101(3):267–273.
3. Amsler M. Kératocõne classique et kératocône fruste; arguments unitaires. *Ophthalmologica* 1946;111(2-3):96–101.
4. Rabinowitz YS, Rasheed K. KISA% index: a quantitative videokeratography algorithm embodying minimal topographic criteria for diagnosing keratoconus. *J Cataract Refract Surg* 1999;25(10):1327–1335.
5. McMahon TT, Szczotka-Flynn L, Barr JT, et al. A new method for grading the severity of keratoconus. *Cornea* 2006;25(7):794–800.
6. Belin MW, Duncan JK. Keratoconus: the ABCD Grading System. *Klin Monbl Augenheilkd* 2016;233(6):701–707.
7. Alió JL, Shabayek MH. Corneal higher order aberrations: a method to grade keratoconus. *J Refract Surg* 2006;22(6):539–545.
8. Alió JL, Piñero DP, Alesón A, et al. Keratoconus-integrated characterization considering anterior corneal aberrations, internal astigmatism, and corneal biomechanics. *J Cataract Refract Surg* 2011;37(3):552–568.
9. Brenner LF, Alió JL, Vega-Estrada A, Baviera J, Beltrán J, Cobo-Soriano R. Clinical grading of post-LASIK ectasia related to visual limitation and predictive factors for vision loss. *J Cataract Refract Surg* 2012;38(10):1817–1826.
10. Scroggs MW, Proia AD. Histopathological variation in keratoconus. *Cornea* 1992;11(6):553–559.
11. Reinstein DZ, Gobbe M, Archer TJ, Silverman RH, Coleman DJ. Epithelial, stromal, and total corneal thickness in keratoconus: three-dimensional display with Artemis very-high frequency digital ultrasound. *J Refract Surg* 2010;26(4):259–271.
12. Reinstein DZ, Archer TJ, Gobbe M. Corneal epithelial thickness profile in the diagnosis of keratoconus. *J Refract Surg* 2009;25(7):604–610.
13. Reinstein DZ, Archer TJ, Urs R, Gobbe M, RoyChoudhury A, Silverman RH. Detection of keratoconus in clinically and algorithmically topographically normal fellow eyes using epithelial thickness analysis. *J Refract Surg* 2015;31(11):736–744.
14. Pircher N, Schwarzhans F, Holzer S, et al. Distinguishing keratoconic eyes and healthy eyes using ultrahigh-resolution optical coherence tomography–based corneal epithelium thickness mapping. *Am J Ophthalmol* 2018;189:47–54.
15. Tang M, Li Y, Chamberlain W, Louie DJ, Schallhorn JM, Huang D. Differentiating keratoconus and corneal warpage by analyzing focal change patterns in corneal topography, pachymetry, and epithelial thickness maps. *Invest Ophthalmol Vis Sci* 2016;57(9):OCT544–OCT549.
16. Li Y, Chamberlain W, Tan O, Brass R, Weiss JL, Huang D. Subclinical keratoconus detection by pattern analysis of corneal and epithelial thickness maps with optical coherence tomography. *J Cataract Refract Surg* 2016;42(2):284–295.
17. Savini G, Schiano-Lomoriello D, Hoffer KJ. Repeatability of automatic measurements by a new anterior segment optical coherence tomographer combined with Placido topography and agreement with 2 Scheimpflug cameras. *J Cataract Refract Surg* 2018;44(4):471–478.
18. Vega-Estrada A, Alio JL, Brenner LF, et al. Outcome analysis of intracorneal ring segments for the treatment of keratoconus based on visual, refractive, and aberrometric impairment. *Am J Ophthalmol* 2013;155(3):575–584.
19. McAlinden C, Khadka J, Pesudovs K. Precision (repeatability and reproducibility) studies and sample-size calculation. *J Cataract Refract Surg* 2015;41(12):2598–2604.
20. Bland JM, Altman DG. Measurement error. *Br Med J* 1996;313:744–753.
21. McGraw KO, Wong SP. Forming inferences about some intraclass correlation coefficients. *Psychol Methods* 1996;1(1):30–46.
22. Reinstein DZ, Yap TE, Archer TJ, Gobbe M, Silverman RH. Comparison of corneal epithelial thickness measurement between fourier-domain oct and very high-frequency digital ultrasound. *J Refract Surg* 2015;31(7):438–445.
23. Temstet C, Sandali O, Bouheraoua N, et al. Corneal epithelial thickness mapping using Fourier-domain optical coherence tomography for detection of form fruste keratoconus. *J Cataract Refract Surg* 2015;41(4):812–820.
24. Li Y, Tan O, Brass R, Weiss JL, Huang D. Corneal epithelial thickness mapping by fourier-domain optical coherence tomography in normal and keratoconic eyes. *Ophthalmology* 2012;119(12):2425–2433.
25. Gokul A, Vellara HR, Patel DV. Advanced anterior segment imaging in keratoconus: a review. *Clin Exp Ophthalmol* 2018;46(2):122–132.