Evaluation of a recently developed noncontact specular microscope in comparison with conventional pachymetry devices

Short title: CCT with noncontact specular microscopy

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The study was performed in accordance with the tenets of the Helsinki Declaration and informed consent was obtained from all subjects.
ABSTRACT

Purpose: The study was conducted to assess the central corneal thickness (CCT) of the healthy cornea with a recently developed noncontact specular microscope (EM-3000; Tomey) and compare the results with those measured with a contact specular microscope and an ultrasound pachymeter. Agreement between measurements taken by 2 investigators was also studied.

Methods: The right eyes of 41 healthy individuals who had negative history of contact lens wear, ophthalmic disease or ocular surgery was examined. The CCT was determined sequentially with a noncontact specular microscope, a contact specular microscope (EM-1000; Tomey) and an ultrasound pachymeter (AL-2000; Tomey). Each evaluation with the specular microscopes were performed by 2 independent operators.

Results: Significant difference was detected in pachymetry measurements among the 3 instruments (p=0.01; Analysis of variance). The mean CCT values were lower measured with the ultrasound pachymeter (537±30 µm) than the contact endothelial microscope (543±37 µm, p=0.17, Student’s t-test) and the noncontact microscope (549±33 µm, p<0.0001) (Operator 1.). There was no statistically significant difference in CCT measurements between the 2 endothelial microscopes (p=0.19). We found significant correlations (p<0.0001) in thickness measurements between each pair of instruments (r=0.91, noncontact microscopy and ultrasound pachymetry; r=0.74, noncontact and contact microscopy; r=0.72, contact microscopy and ultrasound pachymetry; Spearman’s rank correlation).

Conclusions: The strong correlations between the 3 pachymetry devices suggest that the tested instruments provide reliable measurements, however, simply cannot be used interchangeably.

KEY WORDS: agreement, pachymetry, specular microscopy, ultrasound
INTRODUCTION

Central corneal thickness (CCT) is an important parameter to study the effects of intraocular and refractive surgical procedures on the structure and function of the cornea (1-5) as well as to assess the cornea after use of various medications (6). Ocular and systemic diseases can also influence the corneal thickness and its measurements in dry eye (7), keratoconus (8, 9), corneal dystrophies (10), diabetes mellitus (11), glaucoma (12) and contact lens wear (13, 14). The widely performed method of corneal thickness measurement is ultrasonic pachymetry (15, 16), however, several new instruments have been developed, such as confocal microscopy (5, 17, 18), scanning-slit topography (16, 19), interferometry (20), optical coherence tomography (21, 22) and rotating Scheimpflug topography (23, 24). Specular microscopy can determine the corneal thickness and evaluate the corneal endothelium simultaneously.

The purpose of this study was to compare the central corneal thickness measurements obtained with three different pachymeter developed by the same manufacturer (Tomey; Tennenlohe, Germany), a recently introduced noncontact specular microscope (NCSM) (EM-3000; Tomey), a contact endothelial microscope (CSM) and an ultrasound (US) pachymeter.

MATERIALS AND METHODS

Study design

The central corneal thickness was examined in the right eyes of 41 healthy individuals (29 women, 12 men) using a newly developed noncontact specular
microscope and a contact specular microscope (EM-1000; Tomey). The mean age was 45.19±24.11 years (range 19 to 85 years). All subjects had a negative history of ocular disease, trauma or surgery. Contact lens wearers and patients with extensive refractive error (over ± 3.0 D spherical and cylindrical power) were excluded from the study. All procedures adhered to the tenets of the Helsinki Declaration and informed consent was obtained from all subjects.

Each evaluation with the noncontact and contact specular microscopes were performed by two independent investigators. In all patients, measurements were taken sequentially first by noncontact specular microscopy followed by contact microscopy by two operators. Finally, the first investigator determined thickness values with the common standard ultrasound pachymetry instrument (AL-2000; Tomey). To avoid diurnal fluctuation of corneal thickness measurements were taken after 2.0 pm (25).

**Instruments**

For the noncontact specular microscopy, the patient’s chin was positioned on the chin rest and the forehead was pressed against the headband and the person was instructed to look straight ahead. Photos were recorded simply with the auto-alignment and auto-shots functions by pressing the touchscreen and moving the aiming circle to the center of the patient’s pupil until the endothelium was identified. Three photographs were captured by the first investigator. The patient was instructed to remove his/her head from the chin rest and there was a few seconds time interval between image grabbing of the first and the Operator 2. Corneal thickness was determined automatically by the built-in noncontact pachymeter after each examination. The instrument can also evaluate the endothelial cell density and
endothelial morphology.

For the contact specular microscopy, the cornea was anesthetized with topical tetracaine hydrochloride. The probe was disinfected with alcohol after each patient. The person was asked to look at the fixation light and the sequence of the measurements was the same as with the noncontact microscopy. The endothelium was focused, and three images were taken by both operators. The instrument-based software (EM-1100, Version 1.2.2, Tomey) provides an automatic assessment of endothelial cell density and morphology. For corneal thickness, focus values were read from the screen.

Finally, for ultrasound pachymetry, patients were required to look straight ahead and fixate on a target. The operator sat on the left side of the person while the hand-held probe was applied perpendicularly on the center of the cornea. Three pachymetry evaluations were carried out by the first investigator.

In all cases, the average of three measurements was used for further comparative analysis.

**Statistical analysis**

Statistical analysis were performed with the SPSS (Version 9.0.0) and MedCalc Statistical Software (Version 10.0.2.0). Data were described as mean ± standard deviation (SD). Repeated measures analysis of variance (ANOVA) was used for comparison between CCT values measured with the three instruments. To determine the difference between each pair of pachymetry devices Student’s t-test was applied. The correlation between instruments was calculated by Spearman’s rank correlation test, and to describe the relationship between the variables regression analysis was used. Bland-Altman plots were created and the 95% limits of
agreements (95% LoA = mean difference ± 1.96 SD of the difference) were determined to compare the three methods of CCT measurements (26, 27). Intraclass correlation coefficients (ICC2,1) based on the two-way random effects ANOVA, and their 95% confidence interval (95% CI) were calculated to assess interoperator reliability (28). Results were defined as statistically significant if p value was less than 0.05.

RESULTS

Corneal thickness

Pachymetry values obtained with the three instruments are summarized in a table (Tab. I). Lower CCT values were assessed with the ultrasound device than the noncontact and contact specular microscopy (p<0.0001 and p=0.17, respectively) (Operator 1.). ANOVA detected a statistically significant difference in pachymetry measurements among the three instruments (p=0.01, Operator 1.) and there was no significant difference in CCT results between the two types of endothelial microscopes (p=0.19, Operator 1.; p=0.83, Operator 2.). The plots of the difference between each pair of pachymetry instruments against their mean also compare the three devices (Fig. 1, 2 and 3).

There were strong significant correlations in CCT measurements between noncontact and contact specular microscopy (r=0.74, p<0.0001) (Fig. 4), noncontact microscopy and ultrasound pachymetry (r=0.91, p<0.0001) (Fig. 5), and contact specular microscopy and ultrasound device (r=0.72, p<0.0001) (Fig. 6).

Interoperator reliability
CCT measurements assessed by both operators were similar for the noncontact and contact specular microscopy, Student’s t-test did not disclose a statistically significant difference (p=0.05, noncontact; p=0.66, contact) (Tab. II).

Interoperator correlation was excellent for CCT measurements (ICC=0.94, noncontact; ICC=0.90, contact).

DISCUSSION

The present study was performed to compare a recently developed noncontact specular microscope with a contact endothelial microscope and with the common standard ultrasound pachymetry for the thickness measurements of the healthy cornea.

Noncontact specular microscope can auto-focus the corneal endothelium without changing the corneal surface, therefore endothelial image of the noncontact technique is affected by the curvature of cornea (29). Contact specular microscopy evaluates corneal thickness when the light beam is focused on the cornea and the light is reflected from the corneal surfaces with different indices of refraction (air, tear, cornea, and aqueous humor of 1.000, 1.337, 1.376, and 1.336, respectively) (30-34). Contact microscope has an objective cone that applanates the cornea resulting a flat surface where the angle of incidence is equal to the angle of reflection (32, 35).

Ultrasound pachymetry, the widely used technique for CCT measurement (15, 16), is based on the measurement of time interval between echoes of high-frequency sound waves reflected from the anterior and posterior surface of the cornea. The exact posterior reflection point is not known; it may be located between Descemet’s membrane and the anterior chamber (36).
A number of studies have been performed to determine the corneal thickness in normal eyes and in patients with different ocular and systemic diseases. CCT measurements obtained by previous investigators are listed in Table III. These data suggest that our findings are comparable with those in the literature (2, 37, 38). Comparative studies of pachymetry methods have been undertaken to estimate the accuracy and reliability of the different methods. Doughty and Zaman in their meta-analysis of the published CCT results reported a normal average corneal thickness of 535 μm ± 1.96 SD (473 to 597 μm) for Caucasians (10).

Bland-Altman analysis depicts both components of measurement error, i.e. systematic bias (mean difference) between measurements and random error expressed by the width of LoA. In our study, ultrasound pachymetry disclosed statistically significant lower CCT results than the noncontact endothelial microscope, with an acceptable narrow 95% LoA range. The thickest mean CCT value was assessed with noncontact specular microscopy; although the difference between the two microscopes was comparable, the 95% LoA was twice as wide as the LoA range between the noncontact device and ultrasound. These findings suggest that the newly developed noncontact endothelial microscope and the two conventional pachymeters are not interchangeable for corneal thickness evaluation. The possible reasons for this discrepancy can be the distinct imaging principles of the three pachymetry devices as specified above. In addition, both contact specular microscopy and ultrasonic instrument are contact imaging techniques; it can be difficult to place the specular cone (3 mm diameter) and the ultrasonic probe (1.5 mm diameter) at the same location of the cornea (42, 43).

For the association between the three pachymetry methods, there was significant correlation in thickness results between each pair of instruments. Using
the regression analysis, the dependent variable may be predicted from the independent variable. Therefore, regression equations allow the clinician to convert the mean CCT value of one device to those of another one. The different pachymetry instruments to be comparable, use of the following equations is suggested: \( \text{CCT}_{\text{NCSM}} = 207 + 0.63(\text{CCT}_{\text{CSM}}) \) for the noncontact and contact specular microscopes; 
\( \text{CCT}_{\text{NCSM}} = 6 + 1.01(\text{CCT}_{\text{US}}) \) for the noncontact microscope and ultrasound; 
\( \text{CCT}_{\text{CSM}} = 56 + 0.91(\text{CCT}_{\text{US}}) \) for the contact endothelial microscope and ultrasound pachymeter (µm).

The present study evaluated the agreement between two independent investigators and examined operator dependence of the two different specular microscopes. For pachymetry measurements recorded with both noncontact and contact specular microscopy, excellent correlation between the two operators was detected.

There are both advantages and disadvantages of the three instruments. In contrast to ultrasonic pachymetry device, specular microscopes yield additional data on corneal endothelial cell density and morphology. Contact specular microscopy and ultrasonic pachymetry require topical anesthesia before touching the corneal surface. These contact methods are uncomfortable for patients; the ultrasonic probe and the specular cone can cause epithelial lesion and infection. Noncontact specular microscopy is preferred by patients and physicians. Due to the automatic alignment function and pre-installed analysis software, handling of noncontact microscopes is easy. Additional to central corneal measurements, 6 peripherial points of the cornea can be also photographed and evaluated.

In summary, present study compared three pachymetry devices, a recently introduced noncontact specular microscope in comparison with conventional
pachymetry instruments. The noncontact specular microscope measured significantly higher corneal thickness values than the ultrasound pachymeter. There was no significant difference in thickness values between the noncontact and contact specular microscope. Higher degree of correlation between operators was given when using the noncontact endothelial microscope compared with the contact instrument. The strong correlations between the 3 pachymetry devices suggest that the tested instruments provide reliable measurements, however, simply cannot be used interchangeably.
REFERENCES


LEGENDS TO FIGURES

Fig. 1. Bland-Altman plot of difference in central corneal thickness (CCT) assessed with noncontact specular microscopy (NCSM) and ultrasound pachymetry (US) (Operator 1.).

Fig. 2. Bland-Altman plot of difference in CCT measurements assessed with noncontact (NCSM) and contact specular microscopy (CSM) (Operator 1.).

Fig. 3. Bland-Altman plot of difference in CCT results assessed with contact specular microscopy (CSM) and ultrasound pachymetry (US) (Operator 1.).

Fig. 4. Scatterplot combined with regression analysis to show relation between the noncontact and contact specular microscopy in CCT measurements (n=41, r=0.74, p<0.0001, Operator 1.).

Fig. 5. Scatterplot combined with regression analysis to show relation between the noncontact and ultrasound pachymetry in CCT measurements (n=41, r=0.91, p<0.0001, Operator 1.).

Fig. 6. Scatterplot combined with regression analysis to show relation between the contact specular microscopy and ultrasound pachymetry in CCT measurements (n=41, r=0.72, p<0.0001, Operator 1.).