

SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

RECENT ADVANCES IN ANTERIOR SEGMENT IMAGING OF
THE EYE

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INTRODUCTION AND BACKGROUND

Assessment of the anterior segment is an important part of the routine ophthalmological examination, which has traditionally been performed with slitlamp. However, this method cannot provide objective and quantifiable description of the anterior segment structures. The modern imaging techniques have been developing rapidly and novel anterior segment diagnostic instruments promise to overcome these limitations. They are capable of yielding qualitative and quantitative analyses of the cornea, anterior chamber, iris, iridocorneal angle and lens.

The most important parts of the optical system of the eye are cornea and lens, which refract and focus light rays on the retina. The anterior corneal surface has the greatest refractive power due to the high change in refractive index between air and cornea. The endothelium is the fifth and innermost layer of the cornea and consists of a single layer of hexagonal cells. Endothelial cells of the cornea act like active fluid pumps and have barrier function and are responsible for maintaining corneal deturgescence.

Endothelial cell density is an important marker of the corneal health; aging, several disorders, trauma or chemical agents can be associated with endothelial cell loss. *Specular microscopy* allows us for structural and functional non-invasive examination of corneal endothelium. As the light passes through tissues with different refractive indices, some of it can be absorbed by the tissue and some can be reflected. Of primary importance in clinical specular microscopy is the light that is reflected specularly (mirror-like) where the angle of reflection is equal to the angle of incidence. Light reflected from the endothelium-aqueous humor interface is captured by the clinical specular microscope and forms the endothelial image. This provides a base for the corneal endothelial imaging. There are two types of specular microscopes, the contact and non-contact devices.

A wide range of instruments are available for determining corneal thickness, such as ultrasonic and optical pachymetry, anterior segment optical coherence tomography (OCT), scanning-slit topography, partial coherence interferometry (PCI), confocal microscopy, Scheimpflug imaging and specular microscopy. The conventional method of corneal thickness measurement in daily practice is ultrasonic pachymetry, however, several non-contact instruments have become widely practised. *Ultrasound* pachymetry 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 may be located between Descemet's membrane and the anterior chamber. Acute and chronic diseases can affect corneal thickness, including corneal ectasias, glaucoma and diabetes mellitus. This is an important parameter to study the effects of intraocular surgical procedures on the structure and function of the cornea as well as to assess the cornea after use of various medications. Furthermore, corneal pachymetry plays key role in preoperative evaluation of refractive surgery candidates and in risk assessment for ectasia after refractive procedures.

Keratoconus is a non-inflammatory, progressive corneal ectatic disorder that can cause thinning, steepening and bulging of the central cornea. These conditions lead to conical deformity of the normal spherical shape, irregular astigmatism, progressive myopia and vision impairment. Structural and constitutional alterations of the cornea play role in the development of keratoconus. The diagnostic tool for screening corneal ectasias has been developing rapidly with the introduction of several new imaging techniques. Apart from the traditionally applied methods such as slit-lamp examination, keratometry and videokeratoscopy, three-dimensional optical imaging systems have become widely practised, such as scanning-slit tomography, rotating Scheimpflug imaging and anterior segment optical coherence tomography.

Keratometry plays key role in contact-lens fitting, intraocular lens calculation, keratoconus diagnosis and during refractive surgery screening. Traditional *keratometers* evaluate the radius of curvature in two principal meridians at the central 3 mm zone when the anterior corneal surface reflects the projected light like a convex mirror. Keratometers use the first Purkinje-image, which is the reflection of the incident light from the anterior corneal surface. Radius of curvature can be calculated by measuring the size of the object (projected onto the ocular surface), size of the Purkinje-image and distance between the object and Purkinje-image. Then, instruments convert the corneal radius to corneal power with the use of refractive indices of the different refractive surfaces. Keratometers usually use the standardized keratometric index of refraction of 1.3375 to derive the corneal power from the radius of curvature results. *Corneal topographers* projects 25 or 31 concentric Placido-rings onto the ocular surface and the reflected image is captured, digitalized and analysed by the instrument-based software. Videokeratoscopes measure the angle of refraction and its first derivative is the corneal curvature. By evaluating 6400-7936 measuring points, topographers estimate the corneal curvature and shape, and produce the color-coded map of the anterior surface. Measuring corneal curvature is possible either by conventional keratometry or corneal topography, however, in the recent years 3D topography imaging has been introduced into clinical practice, such as scanning-slit and Scheimpflug imaging system. These machines yield data on almost the entire anterior and posterior surface of the cornea, including thickness measurements, and provide data on the anterior segment of the eye.

Pentacam HR (Pentacam High Resolution) is based on the Scheimpflug principle, i.e. when the lens is tilted relative to the film plane, the lens plane, film plane and the plane of focus are not parallel to one another, then the three major planes intersect in a common straight line. Due to this configuration, anterior segment structures (lying in the same plane of focus) from the front corneal surface to the back surface of the lens can be sharply focused

and visualized as tomograms. By use of 475 nm monochromatic (UV-free) blue light, the high resolution camera captures 25, 50 or 100 slit images (i.e. Scheimpflug image) in 2 seconds by rotating along the optical axis from 0° to 360°. After measuring 138 000 true elevation points, the instrument-based software allows for automatic analysis of the anterior segment, anterior and posterior surface topography of the cornea (with axial, instantaneous and elevation maps), pachymetry of the entire cornea, calculation of the chamber angle, chamber height (ACD), chamber volume, the instrument creates a 3D model of the anterior segment and is installed with special image analysis applications.

The recently introduced *swept light source Fourier domain anterior segment OCT* is based on low coherence interferometry. In Fourier domain OCT, light beams reflected by the sample arm and stationary reference arm are combined, and an interference spectrum is detected. The axial image is extracted using the Fourier transform. A swept laser source operating at 1310 nm wavelength can achieve an axial resolution of ≤ 10 microns and a transverse resolution of ≤ 30 microns, and can provide 30 000 axial scans (A-scans) per second. A cross-sectional tomogram (B-scan) can be achieved by laterally combining a series of A-scans, a C-mode image is formed in a plane normal to a B-scan (i.e. parallel to the ocular surface). The software is able to perform automatic analysis of the recorded images providing various corneal maps (axial, instantaneous and refractive corneal power, pachymetry, elevation maps) as well as quantitative and qualitative anterior segment evaluations.

PURPOSE

The diagnostic tool and methods have been developing rapidly; however, the precise theoretical and empirical knowledge of a particular technique is crucial for understanding and interpreting the obtained data. The aim of this study series was to evaluate the reliability and applicability in clinical practice of the novel anterior segment imaging techniques in healthy subjects and in patients with keratoconus, and compare the results to those of the particular reference method.

1. To compare endothelial cell density and morphology between the conventional contact and a recently developed non-contact specular microscopy, and corneal thickness measurements between endothelial microscopy and ultrasound pachymetry. Furthermore, the agreement between two operators and reliability of the two specular microscopes were also tested.

2. To assess the reliability and repeatability of keratometry measurements of the high resolution Scheimpflug camera in healthy subjects, evaluations were taken by two independent investigators. The results of Pentacam HR were compared to those of automated keratometry and corneal topography.

3. To evaluate the repeatability and reliability of a recently introduced swept source Fourier domain anterior segment optical coherence tomography and a high resolution Scheimpflug camera, and to assess the agreement between the two instruments when measuring the anterior segment of healthy individuals and keratoconus patients.

SUBJECTS AND METHODS

Our study series for the dissertation was approved by the local Ethical Committee and all procedures adhered to the tenets of the Helsinki Declaration. Informed consent was obtained from all participants.

1. Non-contact specular microscopy in the evaluation of corneal thickness and endothelium

Subjects. Measurements were performed in the right eyes of 41 healthy individuals (29 women, 12 men) with a mean age of 45 ± 24 years (range 19 to 85 years). All subjects had a negative history of ocular disease, trauma or intraocular surgery. Contact lens wearers and patients with extensive refractive error (over ± 3.0 D spherical and cylindrical power) were excluded from the study. In all patients, pachymetry, endothelial cell density and morphology measurements were taken sequentially first by non-contact specular microscopy (EM-3000; Tomey, Tennenlohe, Germany) followed by contact microscopy (EM-1000; Tomey). Each evaluation with the non-contact and contact specular microscopes were performed by two independent investigators. Finally, the first investigator determined thickness values with an ultrasound pachymetry instrument (AL-2000; Tomey). To avoid diurnal fluctuation of corneal thickness measurements were taken in the afternoon.

Methods. For the non-contact instrument, the person's head was positioned on the chin rest and the subject was asked 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. Some snapshots

were captured by the first investigator. In this study, images from the central cornea were taken for comparative reasons. Then the subject was instructed to remove the head from the chin rest and blink. After a few seconds, the second investigator recorded some additional endothelial snapshots. The best quality picture was selected and analyzed automatically by the pre-installed software of the device. The most important evaluated parameters were the following: endothelial cell density (ECD), average cell area (AVG), coefficient of variation (CV) of cell area, the minimal and maximal cell size. The instrument can also assess the corneal thickness.

For the contact specular microscopy, the cornea was anesthetized with topical tetracaine hydrochloride. The probe was disinfected with alcohol after each subject. The person's head was placed similar to the non-contact device and the subject was instructed to look at the fixation light. The endothelium was focused sharply and three to five images were taken by the first investigator, which were stored on a computer for the further analysis. Then the person was asked to remove the head from the chin rest, and a couple seconds later additional endothelial snapshots were recorded by the second investigator. Both operators analysed their own snapshots with the instrument-based software (EM-1100, Version 1.2.2, Tomey). The frame was set to count as many cells as with the non-contact device. The software assessed the following parameters: endothelial cell density, mean cell size and coefficient of variation. 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 hand-held probe was applied perpendicularly on the center of the cornea under topical anaesthesia. Three pachymetry evaluations were carried out by the first investigator. In all cases, the average of three measurements was used for further comparative analysis.

Specular microscopy evaluates the corneal endothelial cells under magnification. The

magnification changes slightly with corneal thickness as for thinner corneas lower magnification can be expected due to the shorter length of the light path. Thus, the assessed endothelial cell count is supposed to be higher than the real one. In cases of non-contact instruments, magnification also depends on corneal curvature. Therefore, conversion factors were used to correct ECD values. In cases of non-contact devices, conversion table was used as described by Isager et al. For contact specular microscopy, the following equation was applied as provided by the manufacturer: $ECD (corr.) = ECC \times (F / 10.566)^2$, ECD corr. = corrected endothelial cell density, F = image focus (corneal thickness), ECC = estimated endothelial cell count, 10.566 = conversion factor, provided by the manufacturer.

For correction of endothelial cell count measured with the non-contact specular microscope, radius of curvature was assessed with automated keratometry (KR-8100; Topcon, Tokyo, Japan) in every case.

2. Keratometry evaluations with Scheimpflug-camera, automated keratometry and corneal topography

Subjects. Forty-six healthy subjects (30 females, 16 males with a mean age of 50.5 ± 18 years) were recruited. Healthy subjects were defined who had negative history of previous or present ocular and systemic disease. Contact lens wearers were excluded. Corneas with extensive refractive error over ± 4.0 diopters spherical and over 3.0 diopters conventional keratometric astigmatism were also excluded from the study.

Methods. For the Pentacam HR (Pentacam HR; Oculus, Wetzlar, Germany) keratometry evaluations, subjects were asked to place their chin in the chin rest and press their forehead against the forehead strap, and to look at the red fixation target. Anterior segment images were captured automatically by the camera. The keratometry results in flat (Kf) and steep (Ks) meridian at the central 3 mm zone were recorded by analysing axial images.

Immediately after Pentacam HR examinations, automated kerato-refractometry (KR-8100; Topcon, Tokyo, Japan) was carried out in the central 3 mm corneal diameter. Finally, simulated keratometry readings (SIM Kf and SIM Ks) from the central 3 mm diameter zone were measured with corneal topography (TMS-4; Tomey, Erlangen, Germany). Three consecutive measurements were taken with each instrument by both investigators.

3. Reliability and repeatability of swept source Fourier domain optical coherence tomography and Scheimpflug imaging in keratoconus

Patients. Anterior segment evaluations were carried out with a recently developed Fourier domain anterior segment OCT (CASIA SS-1000; Tomey, Erlangen, Germany) and a rotating Scheimpflug tomographer (Pentacam HR; Oculus). 57 participants (32 females, 25 males, mean age: 34 ± 14 years) were normal healthy volunteers (57 right eyes) and 56 patients (84 eyes) (18 females, 38 males, mean age: 32 ± 10 years) have been previously diagnosed with keratoconus. Normal subjects were defined as those with no relevant medical history and with no signs or history of ocular and systemic disease. Contact lens wearers and corneas with extensive refractive error [over ± 4.0 D spherical and over ± 3.0 D keratometric astigmatism] were excluded from the normal group. Patients were diagnosed with keratoconus based on topographic pattern characteristics, as described earlier [steep keratometry (Ks) reading > 48 D; nipple, oval or globus] and slit-lamp findings (corneal stromal thinning, Fleischer ring and Vogt's striae).

Methods. Measurements were taken in 3D mode with the anterior segment OCT and two scan types were used centered at the corneal vertex. "Anterior segment" scan type was used for central corneal thickness and anterior chamber depth measurements which were assessed manually. For anterior and posterior keratometry and pachymetry evaluations in the thinnest and apex position, "Corneal map" scans were recorded and analyzed automatically.

Refractive correction was carried out with the built-in software before the analysis of each "Anterior segment" scan, as suggested previously. The person's chin was positioned on the chin rest and the forehead was pressed against the headband. After choosing the scan type, the person was instructed to blink and to look straight ahead. Scans were recorded simply by moving the aiming circle to the corneal vertex until the focus is properly aligned.

For the Scheimpflug imaging examination, the 25 scans mode was selected and anterior segment images were captured and analysed automatically. The examination was performed as detailed above. The high resolution Scheimpflug system corrects data of the anterior and posterior corneal surfaces for optical and geometrical distortion automatically.

Three consecutive series of anterior segment evaluations (3 anterior segment scan and 3 corneal map radial scans) were made with the anterior segment OCT followed by three scans with a Scheimpflug imaging device. The average of the three measurements was used for further comparative analysis. There was a few seconds time interval between the three repeated measurements for both sets. The axial keratometry results in steep (Ks) and flat (Kf) meridian and astigmatism values at the central 3 mm zone were recorded with both instruments. To derive the corneal power from the radius of curvature results, both the swept source OCT and the Pentacam use the keratometric refractive index of 1.3375 for equivalent total corneal power calculation based on anterior corneal measurements only. Pachymetry measurements were taken in the apex, center of the map, and the thinnest position. Internal anterior chamber depth was determined with both tomographers, i.e. the distance from the posterior corneal surface to the anterior surface of the lens.

Statistical methods

Statistical analysis was performed with the SPSS version 13 (SPSS Inc., Chicago, IL, USA) and MedCalc version 10 (MedCalc Software, Mariakerke, Belgium). Kolmogorov-

Smirnov test was employed to check whether our data were normally distributed. In cases of normal distribution, data were compared using t-test, otherwise non-parametric tests were carried out. Repeated measures analysis of variance (ANOVA) was used to compare the results of 3 instruments. Bland-Altman plots were created to estimate the agreement between the devices, and 95% limits of agreement (LoA) was also yielded as the mean \pm 1.95 standard deviation (SD) of the difference. Within-subject SD (s_w) and repeatability coefficients (CR) for the tested instruments were also calculated using the following equation: $\sqrt{2} \times 1.96s_w$. To assess inter- and intraoperator reliability intraclass correlation coefficients (ICC) and their 95% confidence interval (95% CI) were calculated. A P value below 0.05 was considered statistically significant.

RESULTS

1. Non-contact specular microscopy in the evaluation of corneal thickness and endothelium

Endothelial cell density. Both raw and corrected ECD measurements obtained with the non-contact instrument were significantly higher in comparison with the contact microscope by both investigators ($P < 0.0001$). The difference between raw and corrected ECD was +10 cells/mm² with the non-contact and -63 cells/mm² with the contact instrument. The mean difference in corrected ECD was $452 \pm 196 / 452 \pm 215$ cells/mm² (with a LoA of 836 to 67 / 873 to 30 cells/mm²) between the microscopes. There was a significant correlation ($P < 0.0001$) in ECD measurements ($r = 0.773$, first operator; $r = 0.635$, second operator) between the specular microscopes.

Average cell area and coefficient of variation. In the measurements of AVG and CV of cell area there were statistically significant differences between the two endothelial microscopes ($P < 0.0001$). Higher values of both morphologic parameters were observed with the contact instrument by each investigator. The mean difference in AVG measurements was $58 \pm 30 / 60 \pm 34$ μm^2 (with a LoA of -1 to +118 / -7 to +126 μm^2). For the CV of cell size, the difference between the two microscopes was $12 \pm 9 / 13 \pm 11$ (with a LoA of -5 to +29 / -8 to +34). Spearman's rank correlation test disclosed a statistically significant correlation ($P < 0.0001$) in AVG measurements between the contact and non-contact microscopy ($r = 0.718$, first operator; $r = 0.640$, second operator). There was no significant association in CV of cell area results determined with the two instruments for either operator ($r = 0.247$, $P = 0.120$, first operator; $r = 0.141$, $P = 0.379$, second operator).

Central corneal thickness. Lower pachymetry values were assessed with the ultrasound device than with the non-contact and contact specular microscopy ($P < 0.0001$ and $P = 0.170$, respectively). The highest pachymetry values were recorded with the non-contact device. ANOVA detected a statistically significant difference in pachymetry measurements among the three instruments ($P = 0.01$) and there was no significant difference in thickness results between the two types of endothelial microscopes ($P = 0.190$, first operator; $P = 0.380$, second operator). There were significant correlations ($P < 0.0001$) in pachymetry measurements between non-contact and contact specular microscopy ($r = 0.739$), non-contact microscopy and ultrasound pachymetry ($r = 0.912$), and contact specular microscopy and ultrasound device ($r = 0.715$). Interoperator correlation was excellent for corneal thickness measurements ($ICC > 0.90$).

Agreement between operators. Neither in ECD values nor in other morphologic parameters were statistically significant differences between the two operators ($P > 0.05$). Central corneal thickness assessed by both operators were similar for the non-contact and contact specular microscopy, Student's t-test did not disclose a statistically significant difference ($P = 0.051$, non-contact; $P = 0.660$, contact). Although the mean interoperator difference in ECD results obtained with the non-contact and contact microscopes was similar (9 ± 100 cells/mm² and 9 ± 125 cells/mm²), the wider LoA for the contact device (-254 to $+236$ cells/mm²) indicated a greater variability when compared to the non-contact instrument (95% LoA of -205 to $+188$ cells/mm²). For AVG and CV of cell area values, also higher interoperator difference and variability were measured with the contact specular microscope. For endothelial cell density and cell size, higher correlation coefficients were found ($ICC > 0.94$) with the non-contact microscope, but good agreement was observed with the contact instrument ($ICC > 0.81$). For variation of coefficient of cell size measurements, ICC value

were below 0.75, higher correlation was detected with the non-contact (ICC = 0.72) than the contact endothelial microscopy (ICC = 0.35).

2. Keratometry evaluations with Scheimpflug-camera, automated keratometry and corneal topography

Difference between devices. The mean keratometry values obtained with the Pentacam HR, automated keratometry and corneal topography were 43.40 ± 1.49 D / 43.34 ± 1.40 D, 43.99 ± 1.42 D / 43.98 ± 1.44 D and 43.80 ± 1.38 D / 43.83 ± 1.36 D, respectively by investigator 1 and 2. Keratometry in flat and in steep meridians taken with the Pentacam HR were significantly lower ($P < 0.0001$, repeated-measures ANOVA) when compared to the Kf and Ks values of automated keratometry or corneal topography. The differences between the measurements of the rotating Scheimpflug camera and the corneal topography were lower both for Kf (0.30 ± 0.67 D, $P = 0.004$, investigator 1; 0.33 ± 0.68 D, $P = 0.002$, investigator 2, post hoc Tukey test) and for Ks values (0.51 ± 0.75 D, $P < 0.0001$, investigator 1; 0.67 ± 0.73 D, $P < 0.0001$, investigator 2) than the differences between the Pentacam HR and the automated keratometry results (Kf: 0.60 ± 0.54 D, $P < 0.0001$, investigator 1; 0.63 ± 0.50 D, $P < 0.0001$, investigator 2; Ks: 0.60 ± 0.58 D, $P < 0.0001$, investigator 1; 0.66 ± 0.69 D, $P < 0.0001$, investigator 2).

Repeatability. There were no statistically significant differences between 3 consecutive measurements of either instrument ($P > 0.05$). Based on within-subject SD values, the variability between repeated measurements on the same subject was the highest using the Pentacam and the lowest using the automated keratometer. The calculated repeatability coefficients were 0.79 D / 1.55 D (Kf) and 1.02 D / 2.06 D (Ks) for the Pentacam HR, 1.01 D / 0.28 D (Kf) and 0.73 D / 0.54 D (Ks) for the automated keratometer, 1.25 D / 1.08 D (Kf) and 1.26 D / 0.97 D (Ks) for the corneal topographer.

Reliability. The reliability of the automated keratometry was the highest (intraoperator ICC ranged from 0.97 to 0.99) when compared to the Pentacam (intraoperator ICC ranged from 0.80 to 0.97) or to the corneal topography (intraoperator ICC ranged from 0.91 to 0.95). Statistical analysis did not disclose significant differences between the keratometry parameters of the 3 instruments obtained by the 2 operators ($P = 0.215-0.983$, paired t-test). The Pentacam HR showed good interoperator reliability (ICC = 0.97 / 0.95) for K readings; however, the automated keratometry (ICC = 0.99 / 0.97) and corneal topography (ICC = 0.98 / 0.97) measurements indicated better agreement between the 2 investigators reflected by the interoperator ICC values.

3. Reliability and repeatability of swept source Fourier domain optical coherence tomography and Scheimpflug imaging in keratoconus

Difference between healthy and keratoconus subjects. Significant differences were observed in all mean measured anterior segment parameters between the normal and keratoconus groups ($P < 0.0001$, Mann-Whitney U test) for Fourier domain OCT and Pentacam HR.

Difference between devices. In the normal group, significant differences between the tested machines were established in the K values, pachymetry and ACD results ($P > 0.05$, Wilcoxon-test). In keratoconus patients, every measured parameter differed significantly between the two instruments except the front astigmatism and posterior Ks results. In the keratoconus group, higher difference with a wider LoA was found in most measured parameters between the two tomographers when compared to the normal individuals.

Repeatability. Statistical analysis did not reveal significant difference in any of the measured anterior segment parameters between the three consecutive evaluations of either the anterior segment OCT ($P = 0.062 - 0.70$, normal subjects; $P = 0.113 - 0.979$; keratoconus

patients; ANOVA) or the rotating Scheimpflug system ($P = 0.06 - 0.815$, normal subjects; $P = 0.207 - 0.783$; keratoconus patients). In the healthy group, the difference between repeated measurements was less for the OCT than for the Scheimpflug imaging in the posterior Ks, Kf and astigmatism values, and in the apical pachymetry results. On keratoconic eyes, the difference between the three measurements was less for the anterior segment OCT in every keratometry and astigmatism value, in apical thickness and ACD results. Repeatability was higher on healthy corneas than on keratoconic eyes for every keratometry value, the thinnest and center pachymetry measurements obtained with both instruments.

Reliability. In the normal group, reliability was better for the back keratometry and astigmatism values, ACD and apical pachymetry measurements evaluated with the anterior segment OCT than with the Scheimpflug system. In the keratoconus cases, OCT provided more reliable K, keratometric astigmatism and apical corneal thickness results when compared to the high resolution Scheimpflug tomographer.

DISCUSSION

1. Non-contact specular microscopy in the evaluation of corneal thickness and endothelium

Endothelial cell density is an important marker of the corneal health; normal aging, several disorders or trauma can be associated with endothelial cell loss. Contact specular microscopy is widely used for studying the endothelium in vivo by applanating the corneal surface. In contrast, the recently developed non-contact instrument can easily evaluate the endothelial layer of the cornea without touching the eye.

The present study was performed to compare the two types of specular microscopes for the measurement of endothelial cell density and morphology of the healthy cornea. Both non-contact and contact endothelial microscopes operated with their instrument-based analysis softwares and used the fixed frame method of cell counting. We found statistically significant higher ECD values assessed with non-contact microscopy, the difference between the two specular microscopes was remarkable. The cause of this difference could either be linked to the distinct operating principles of the two instruments or to discrepancies in the output from the images analysis software programs on the two instruments. While it has been noted that differences in the optical principles could alter the final image size, such effects can be expected to be small. The non-contact specular microscope can auto-focus the corneal endothelium without changing the corneal surface, therefore endothelial imaging is also affected by the curvature of cornea. The contact microscope has an objective cone that applanates the cornea resulting in a flat surface where the angle of incidence is equal to the angle of reflection. The contact cone may displace or compress the precorneal tear film; therefore, the light passes through only the corneal layers. In cases of non-contact methods,

two additional refractive media (i.e. air and tear film) may affect the refraction and the specular image. The likely reason for the discrepancy between the measurements from the two instruments is in the image analysis software and the way it was used. The two software options do not equally identify the cell borders. The result is that, for the contact microscope's image software, a few or even many cells are reported to have erroneously larger areas. As with any such errors, this will result in higher mean cell area (and thus lower ECD) and higher coefficient of variation values being obtained. Higher difference and variability between two investigators was detected with the contact instrument.

In our study, ultrasound pachymetry disclosed statistically significant lower central corneal thickness results than the non-contact endothelial microscope, with an acceptable narrow 95% LoA range. The thickest mean central thickness value was assessed with non-contact specular microscopy; although the difference between the two microscopes was comparable, the 95% LoA was twice as wide as the LoA range between the non-contact device and ultrasound. 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. For the association between the three pachymetry methods, there was significant correlation in thickness results between each pair of instruments. For pachymetry measurements recorded with both specular microscopes, excellent correlation between the two operators was detected.

Doughty and Zaman in their meta-analysis of the published central corneal thickness results reported a normal average corneal thickness of $535 \mu\text{m} \pm 1.96 \text{ SD}$ (473 to 597 μm) for Caucasians. According to our findings, M6dis et al., Nichols et al. and Tam et al. also

detected lower pachymetry measurements with ultrasound device in comparison with specular microscopy.

In summary, our results indicated significant difference and poor agreement between the tested endothelial microscopes and their automated image analysis programs. Based on our data, the two diagnostic instruments cannot be used interchangeably in clinical practice, and thus for patient follow-up one certain device is recommended. Statistically significant difference was observed in central corneal thickness measurements between the non-contact microscopy and ultrasound pachymetry. The strong correlations between the 3 pachymetry devices suggest that the tested instruments provide reliable measurements, however, simply cannot be used interchangeably.

2. Keratometry evaluations with Scheimpflug-camera, automated keratometry and corneal topography

The present study compared automated keratometry and corneal topography measurements to the keratometry results of the Pentacam HR imaging system. In healthy corneas, significantly lower dioptric power values were obtained with Scheimpflug tomography by both investigators in comparison with the two conventional devices, although the differences between them were only 0.3 - 0.7 D. Similar to our results, previous authors also reported lower K values measured with Pentacam, than with corneal topography or automated keratometry. Kawamorita et al. found significant difference between Pentacam and topography (K1: -0.44 ± 0.37 D; K2: -0.26 ± 0.41 D, $P < 0.01$). In another study, authors described irrelevant difference between Pentacam and automated keratometry (difference between mean K values: -0.046 D) but the range between limits of agreement was broader than our results (95% LoA ranging from -1.32 D to $+1.23$ D).

These differences may derive from the distinct operating principles of the keratometry devices. Conventional keratometers evaluate the radius of curvature in two meridians at the central 3 mm diameter zone, which covers only 6% of the anterior corneal surface. Videokeratoscopes provides data on more than 60% of the anterior surface but cannot image the posterior corneal surface. Keratometers and corneal topographers usually use the standardized keratometric index to derive the corneal power from the radius of curvature results. Whereas the Scheimpflug camera captures 25, 50 or 100 slit images by rotating along the optical axis from 0° to 360° and evaluates real height data and the true corneal shape. The instrument derives curvature and refractive power values from elevation, and provides pachymetry maps of the entire cornea by using the elevation data of both anterior and posterior surfaces. Corneal curvature values and maps depend on the measurement reference axis. The Placido-based topographer uses the corneal vertex normal (V K axis) as a reference axis when the optical plane of videokeratograph is normal to the corneal surface. The Scheimpflug camera captures slit images by rotating along the optical axis, when the corneal shape is maximally symmetric in all six meridians.

In the present study, keratometry data did not demonstrate significant interobserver differences either with the Pentacam HR or with other machines. The strong positive correlation between the tested keratometry devices indicated that the measurements of each technique are reliable. Although all keratometry devices provided reliable measurements, the strongest correlation between repeated evaluations was found with automated keratometry. The lowest variability between consecutive measurements was observed in cases of automated keratometry in comparison with the Pentacam HR and corneal topography.

The present study established statistically significant differences in keratometry results between the high resolution Pentacam, automated keratometry and corneal topography systems. The keratometry measurements of each technique were reliable and repeatable.

However, we found automated keratometry to be the most repeatable and reliable method both for flat and for steep K evaluations emphasizing the gold standard role of the traditional method in keratometry evaluation. Pentacam HR, automated keratometry and corneal topography cannot be used interchangeably in clinical practice due to the significant difference between the 3 keratometry devices.

3. Reliability and repeatability of swept source Fourier domain optical coherence tomography and Scheimpflug imaging in keratoconus

. Swept source OCT is a recent development of Fourier domain OCT systems using a wavelength-tuned laser source. There are several advantages of swept source OCT over time domain or spectral domain OCT devices. Swept source OCT is faster (with an image acquisition time of 0.3 second in "Corneal map" mode and 0.2 – 4.8 seconds in "Anterior segment" mode) than former anterior segment OCT systems reducing the effect of motion artifacts. Apart from the high axial and lateral resolution (≤ 10 microns and ≤ 30 microns, respectively), swept source OCT is also capable of performing large depth scans with 6 mm tissue penetration and 16 x 16 mm horizontal and vertical scan ranges. Due to the 1310 nm wavelength laser source, incident light can be absorbed by water improving the safety profile of the examination (less than 6% of light energy reach the retina) and allowing deeper scleral penetration and a more precise imaging of the irido-corneal angle structures. Optical coherence tomography is thought to achieve a higher resolution (i.e. few-micron) than computer tomography or magnetic resonance imaging; therefore, this non-invasive technique is also referred to optical biopsy.

In our study, significant differences were calculated in all measured anterior segment parameters between the normal and keratoconus group obtained with both OCT and high resolution Scheimpflug camera device. This finding is consistent with those reported in the

literature. In both subject groups, the difference between the three consecutive measurements performed on the same individual was less for the anterior segment OCT than for the Scheimpflug system in back steep, back flat keratometry and astigmatism values, and apical corneal thickness results. Li et al. reported similar within-subject SD (6.9 μm and 5.5 μm , respectively), repeatability (13.6 μm and 10.8 μm) and ICC values (0.96 and 0.98) to our findings when corneal thickness was measured manually. For keratometry and astigmatism measurements, repeatability was better on healthy corneas than on keratoconic ones assessed with both instruments; however, this tendency was not observed for every pachymetry value. Since central corneal thickness and ACD were assessed manually with the OCT and automatically by the Scheimpflug camera device, this could possibly affect the repeatability of the two instruments. Moreover, six initial anterior segment evaluations were performed with the OCT on every individual, thus both the operator and the subjects could be more tired during the Scheimpflug examination which might also worsen the repeatability performance of the latter method. In a previous comparative study, authors reported higher repeatability of central corneal thickness measurements with Visante OCT assessed both in normal (repeatability coefficient ranging from 4.63 to 7.68 μm) and in keratoconus groups (repeatability coefficient ranging from 7.17 to 15.24 μm), although ICC values (ICC > 0.90 in both groups) were similar to those obtained in our study.

In this study, both components of the measurement error associated with the instrument were considered. Systematic bias was assessed by the mean difference between measurements and random error was determined by the width of LoA. The mean difference between anterior segment OCT and high resolution Scheimpflug imaging was generally higher with a wider LoA in patients with keratoconus when compared to the normal group. However, a recent study using the prototype of swept light source anterior segment OCT and a Scheimpflug imaging instrument demonstrated higher difference in central pachymetry

values and lower difference in ACD measurements on healthy corneas than those observed in our study.

Based on our results, the potential clinical significance of these devices to detect keratoconus includes the following: pachymetry measurements of the entire cornea, true elevation data and topography maps of the anterior and posterior corneal surfaces. In K values, pachymetry and ACD, statistically significant differences were detected between the anterior segment OCT and the rotating Scheimpflug tomographer. This difference might be attributed to the distinct optical principles and calculation methods of the two instruments as mentioned above. However, Bland-Altman plots revealed good agreement between the measurements of the two devices. Both instruments provided reliable anterior segment evaluations on normal and keratoconic eyes, and repeatability of their measurements was comparable.

SUMMARY OF NEW RESULTS AND THEIR CLINICAL RELEVANCE

1. Our findings emphasize that software options of the tested contact and non-contact specular microscopes do not equally identify the endothelial cell borders in healthy corneas. The result is that, for the contact microscope image software, a few or even many cells are reported to have erroneously larger areas and to be more irregular in shape. As with any such errors this will result in higher average cell size and thus lower cell density, and higher coefficient of variation values being obtained. We also detected higher difference and greater variability between the measurements of the two operators when using the contact method. The non-contact specular microscope measured significantly different corneal thickness values than the ultrasound pachymeter; however, strong correlations were observed between the 3 pachymetry devices.

2. Our findings suggested that keratometry measurements of the high resolution Pentacam, automated kerato-refractometry and corneal topography were reliable and repeatable. However, the strong correlation between two independent investigators and repeated measurements on the same subject indicated that automated keratometry was the most repeatable and reliable method. Therefore, conventional automated keratometry is considered to be the gold standard for evaluating corneal power.

3. Both the Fourier domain anterior segment OCT and the rotating Scheimpflug-camera can provide high resolution anterior segment scans as well as automatic analysis of the recorded images. Based on our findings, these properties seem to be beneficial not only in the examination of healthy corneas, but these advances become more pronounced in keratoconus. Both imaging systems produced reliable and repeatable anterior segment analyses in healthy and in keratoconus patients, which emphasizes a considerable role of both tomographers in screening and following patients with keratoconus.

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List of publications related to the dissertation

1. Módis jr., L., **Szalai, E.**, Kolozsvári, B., Németh, G., Vajas, A., Berta, A.: Keratometry evaluations with the pentacam high resolution in comparison with the automated keratometry and conventional corneal topography.
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