Comparison of Central Corneal Thickness Measured by Standard Ultrasound Pachymetry, Corneal Topography, Tono-Pachymetry and Anterior Segment Optical Coherence Tomography

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Comparison of Central Corneal Thickness measured by Standard Ultrasound Pachymetry, Corneal Topography, Tono-Pachymetry and Anterior Segment Optical Coherence Tomography.

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Running Head Title: Reliability of Ultrasound and 3 non-contact pachymeters

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Key-words: Central corneal thickness, ultrasound pachymetry, non-contact tono-pachymetry, Scheimpflug camera, anterior segment optical coherence tomography, interchangeability.

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Abstract

**Purpose:** To compare central corneal thickness (CCT) measured by standard ultrasound pachymetry (USP), and three non-contact devices in healthy eyes.

**Methods:** A cross-sectional study of CCT measurement in 52 eyes of 52 healthy volunteers was done by a single examiner at Ocular Surface and Contact Lens Laboratory. Three consecutive measurements were done by standard USP, non-contact tono-pachymeter, Pentacam corneal topographer, and Anterior Segment Optical Coherence Tomography (AS-OCT). The mean values were used for assessment. The results were compared using multivariate ANOVA, linear regression and Pearson correlation. Agreement among the devices was analyzed using mean differences and Bland-Altman analysis with 95% limits of agreement (LoA). Finally, reliability was analyzed using intraclass correlation coefficient (ICC).

**Results:** Mean CCT by ultrasound pachymeter, tono-pachymeter, corneal topographer and AS-OCT were 558.9±31.2 µm, 525.8±43.1 µm, 550.4±30.5 µm and 545.9±30.5 µm respectively. There was a significant positive correlation between AS-OCT and USP (Pearson correlation = 0.957, p < 0.001), corneal topography and USP (Pearson correlation = 0.965, p < 0.001) and corneal topography and AS-OCT (Pearson correlation = 0.965, p < 0.001). There was a lower correlation between CT-1P tono-pachymeter and the other three modalities. Intraclass correlation coefficients show an excellent reliability between pairs except for CT-1P against the other three instruments that were found moderate.

**Conclusions:** CT-1P tono-pachymeter underestimates CCT measurements compared to Scheimpflug system, AS-OCT device, and USP. Mean CCT among USP, Pentacam and AS-OCT were comparable and had significant linear correlations. In clinical practice, these three modalities could be interchangeable in healthy patients.
Introduction

Central corneal thickness (CCT) is an important and sensitive indicator of corneal health.\textsuperscript{1} It is necessary in monitoring corneal diseases such as corneal oedema, keratoconus, Fuchs dystrophy, glaucoma and to evaluate corneal barrier and endothelial pump function in several surgical conditions.\textsuperscript{2-4} In clinical practice, it is useful in the evaluation of contact lens wear,\textsuperscript{5,6} selecting patients for refractive surgery and posterior evaluation.\textsuperscript{7,8}

CCT is also a predictive factor for glaucoma progression in patients with high baseline intraocular pressure (IOP). Moreover, CCT is an important parameter in the risk profiling of ocular hypertensive to glaucoma patient.\textsuperscript{9,10} Since IOP measurement by applanation tonometry is influenced by CCT, it is important to obtain the reliable corneal pachymetry for each patient and adjust the IOP for the measured CCT.\textsuperscript{1,11}

There are numerous methods available to measure CCT. Ultrasound pachymetry (USP) has been widely considered as the gold standard because it is very easy, fast and convenient to repeat several measurements to minimize error.\textsuperscript{12,13} USP requires contact with the cornea and uses the Doppler Effect to determine CCT. Disadvantages of ultrasonic pachymetry include direct placement of the probe on the cornea, the risk of infection and corneal epithelial damage, the necessity for topical anesthesia (which may influence by up 10 microns CCT measurements), and dependence on examiner experience for reliable measurements.\textsuperscript{14-15}

Optical Coherence Tomography (OCT), which was introduced in the early 1990s, is a noncontact imaging method that provides detailed cross-sectional images of biological tissues by measuring their optical reflections.\textsuperscript{16,17} OCT has been widely used clinically
in ophthalmologic practice for the last two decades. In recent years, OCT technology has experimented the incorporation of spectral-domain (SD) imaging that offers significant advantages over the traditional time-domain (TD) techniques, which include faster imaging speed, higher resolution, and better visualization. Simultaneously with these improvements, the utility of OCT in the ophthalmic practice has become more extended. Particularly, anterior segment OCT (AS-OCT), which provides high-resolution cross-sectional images of anterior segment structures, including corneal thickness, anterior chamber angle, conjunctiva, and tear meniscus, has recently gained popularity. There are very few studies giving comparative accuracy of CCT measurements by AS-OCT versus USP. The Pentacam, developed in 2000s, uses a rotating Scheimpflug camera and a slit-light source that rotate together around the optical axes of the eye to calculate a three-dimensional model of the anterior segment. A total of 25 images are captured within 2 s, with each slit image composed of 25,000 points including 500 true elevation points. As a pachymeter, Pentacam provides a corneal thickness map and determines the thinnest point as well. Previous studies have shown that Pentacam has high agreement compared with USP, high intraoperator repeatability and reproducibility for CCT measurements. In recent years, several units of non-contact tonometry and pachymetry have been developed. Tono-pachymetry simultaneously measures CCT using the principle of the Scheimpflug camera system and IOP using a conventional non-contact tonometry method. Tono-pachymetry is patient-friendly and time-saving, but it has not been well documented whether the CCT values obtained from tono-pachymetry are comparable to those derived from conventional USP as the gold standard for measuring CCT. To the best knowledge of the authors, this was one of the few studies that was designed to compare the correlation and agreement between CCT measurements.
obtained using recently marketed, CT-1P tono-pachymetry, 3D OCT-2000 and Pentacam with USP in young myopic healthy eyes.

**Materials and Methods**

**Study Design and subjects:** This prospective cross-sectional comparative study includes 52 eyes of 52 healthy subjects voluntarily enrolled at the Ocular Surface and Contact Lens Laboratory (LSOYLC) from the University of Santiago de Compostela. All subjects after CCT measurement were subjected to a full ophthalmic examination. This examination included routine evaluation of visual acuity, refractive error and slit lamp biomicroscopy with particular attention to the presence of ocular adverse events. The inclusion criteria were age between 18-30 years, normal corneal topographic pattern, myopia between -6.00 D and -0.75 D, no more than -1.75 D of astigmatism, correctable to 20/20 and also included emmetropic eyes achieving 20/20 or better visual acuity. Exclusion criteria included previous refractive surgery, corneal diseases, recent use of contact lenses, no other systemic or ocular diseases, and use of topical medications.

The study was performed according to the renewed and revised rules of Helsinki Declaration and was approved by the Ethics Committee of the University of Santiago de Compostela.

**Technologies used to measure CCT:** Three consecutive measurements were done by standard USP, non-contact tono-pachymeter, corneal topography, and anterior segment optical coherence tomography (AS-OCT). In order to eliminate effects of diurnal variation on thickness, all measurements were taken between 2 PM and 6 PM.30
Automatic analysis by Scheimpflug camera Pentacam (Oculus Optikgerate GmbH, Wetzlar, Germany) was performed for all eyes. Multiple slit images of the anterior segment with 500 true elevation points are captured by the rotating camera. CCT was recorded only when the examination quality specification reading was satisfactory; otherwise it was excluded and reanalyzed until three valid readings were obtained.

Spectral-domain optical coherence tomographic 3D OCT-2000 (Topcon, Tokyo, Japan) equipment was used for anterior segment analysis using the headrest attachment. It captures high resolution images of the cornea using non-contact OCT, allowing for topographical mapping of the cornea including corneal thickness. The system obtained different images, separated by 0.25 mm with 5-6 micron of axial resolution and 20 microns of transverse resolution. The corneal thickness was measured by an automated algorithm, that detects epithelium and endothelium limits on the cross-sectional images of the cornea. The mean value from three consecutive measurements was taken as the CCT value.

The non-contact tono-pachymeter CT-1P (Topcon, Tokyo, Japan), also was used for pachymetric analysis by a specular microscope method. The patient was seated and asked to look at a fixation target. The emitted light from a narrow slit in the cornea is reflected by the front and backside of the cornea and CCT was measured according to the interval between both reflection images on the line sensor. The operator visualized a real-time image of the patient’s eye on the screen. Although the operator manually focused the image to the center of the pupil, CT-1P tono-pachymeter automatically measured CCT three times and calculated the average value.

These non-contact measurements were followed by USP (Paxis, Biovision Inc, Clermont-Ferrand, France). The subject was seated on the chair and asked to look at fixation light located straight ahead. The examiner placed the pachymeter probe on the
central cornea as perpendicular as possible. Three consecutive measurements were taken by the same experienced examiner and the average was recorded for each patient. USP was performed under topical anesthesia with tetracaine hydrochloride 0.5% (Colircusí Anestésico, Alcon Cusí, Barcelona, Spain).

Data Analysis: Data were analyzed by SPSS version 20 (SPSS Inc., Chicago, Illinois, USA) using descriptive statistics, linear regression and Pearson correlation coefficient. The CCT measured by USP and the three non-contact devices was compared using multivariate analysis of variance (ANOVA), and pairwise comparisons were performed using the Bonferroni adjustment for multiple comparisons. A p-value of less than 0.05 was considered statistically significant. Bland-Altman plot was used to evaluate the agreement between the four techniques. Finally, reliability was analyzed using intraclass correlation coefficient (ICC). Based on the 95% confident interval of the ICC estimate, values less than 0.5, between 0.5 and 0.75, between 0.75 and 0.9, and greater than 0.90 are indicative of poor, moderate, good, and excellent reliability, respectively.31-32

Results

A total of 52 eyes in 52 healthy subjects (only right eyes) were studied (32 females and 20 males). The mean age was 23.52±3.78 years (range 20 to 28 years) and a mean spherical equivalent of -1.56±1.78 D (range -0.50 to -5.75 D).

The highest CCT mean value was obtained with the USP (558.9 ± 31.2 µm; range from 476 to 614 µm), followed by the Pentacam (550.4±30.5 µm; range from 465 to 615 µm), then by the 3D OCT (545.9±30.5 µm; range from 457 to 602 µm), and finally, the
The lowest value was obtained with the tono-pachymeter (525.8±43.1 µm; range from 431 to 674 µm).

**Figure 1** shows a significant positive correlation between the CCT readings obtained by USP and by the non-contact devices. There was a significant strong correlation between AS-OCT and USP (Pearson correlation = 0.979, p < 0.001), Pentacam and USP (Pearson correlation = 0.946, p < 0.001) and Pentacam versus AS-OCT (Pearson correlation = 0.951, p < 0.001). The correlation coefficient between tono-pachymeter and the other three modalities was significantly lower than between USP, Pentacam and OCT with each other.

The ANOVA analysis (**Table 1**) showed statistically significant differences between CCT mean values from all paired instruments (p < 0.001 in all cases). The highest difference between pairs was found between tono-pachymeter CT-1P and USP (-33.1 ± 33.3 µm; CI -15.2, 8 to 50.9 µm; p < 0.001), while the lowest difference was found between OCT and Pentacam (-4.5 ± 9.5 µm; CI 13.3 to 22.4; p = 0.001) but followed too close by difference between Pentacam and USP (-8.5 ± 10.2 µm; CI 9.4 to 26.4; p < 0.001) or between OCT and USP (-13.0 ± 6.4 µm; CI 4.9 to 30.9; p < 0.001).

Bland-Altman analysis confirmed these results, CCT obtained by Pentacam, 3D OCT-2000 and USP pairs showed excellent agreement, with the mean difference centered close to zero, and 95% of the points were accurately located between the predicted 95% limits of agreement (**Figure 2ABC**). Conversely, tono-pachymeter CT-1P showed the lowest concordance when compared with USP, Pentacam or OCT (**Figure 2DEF**), with the higher differences (mean ± 1.96 SD). The limits of agreement 95% (LoA = mean of the difference ± 1.96 × SD of the differences) indicates that the values on the error between the pairs of measurement have exceeded the limits of concordance. Particularly, tono-pachimetry underestimated CCT by 33.1 µm when compared with
USP, with 95% LoA ranging between 23.8 and 42.3 µm. The plot indicates that the difference between tono-pachimetry and the other methods decreased significantly (r = 0.39; r = 0.44; r = 0.43 p < 0.01), showing lower CCT (proportional bias) for thinner corneas and moving to higher CCT when measuring thicker corneas (Figure 2DEF).

For a more complete reliability analysis between pairs of CCT measurements, the ICC values was calculated and can be seen in Table 1. The reliability between all pairs was statistically significant (p < 0.001).

**Discussion**

According to our findings, the average values of CCT taken with the four instruments were significantly different in healthy myopic patients. Our data demonstrated that Pentacam, AS-OCT and CT-1P tono-pachymetry significantly underestimates CCT compared with the USP, considered as the gold standard, by -8.5 ± 10.2 µm, -13.0 ± 6.4 µm and -33.1 ± 33.3 µm respectively. Several studies demonstrated that Scheimpflug-base system, as Pentacam, significantly underestimates CCT compared with USP in myopic patients before and after LASIK.\(^{33,34}\) Conversely, other authors found that Pentacam tends to overestimate CCT compared to USP after LASIK.\(^{35,36}\) Other studies have analyzed the relationship between different spectral domain AS-OCT devices from other manufactures and, in most of those papers difference between OCT and USP measurement was similar to the differences shown in this research.\(^{37-40}\) There are reports with different tono-pachimetric devices, using a different operating principle (the Scheimpflug-base system), showing an underestimation of CCT when compared with USP.\(^{40-42}\) However, the underestimation reported by these authors was less than difference observed in our study. Sagdik et al, also found that mean CCT was 28.4 µm thinner than USP using the CT-1P device, to the best of our knowledge the
unique study found using the same tono-pachymetry system.\textsuperscript{43} Moreover, we found a trend towards larger differences for thinner corneas and lower differences for thicker corneas, despite showing a moderate correlation for the difference versus mean in the Bland-Altman analysis, this is not clinically relevant.

There are many possible reasons to explain these differences, in part derived from the different operating principles of each instrument. Factors conditioning USP measurements include decentration, oblique incidence of the probe to the cornea, and the necessity for topical anesthesia, which may influence by up 10 µm CCT measurements.\textsuperscript{14-15} However, apart from the much training of the operator, perpendicularity of the probe (if present) would not induce significant error as the probe is indeed quite sensitive to alignment errors. If the probe misaligns by 10° or more, the reading is not done because the “eco” is not captured by the receptor. In contrast, the indentation of the cornea by direct placement of the probe and displacement of the tear film can lead to underestimation of CCT with increased risk of corneal epithelial damage. Moreover, reliability may be influenced by variability of ultrasound speed in tissues of different hydration and dependence on examiner experience.\textsuperscript{44-45} Conversely the main advantage of the new non-contact measuring systems is that they avoid contact with the cornea, eliminating the risk of edema or epithelial damage. New AS-OCT systems include faster imaging speed (nearly 26,000 A-scans per second), higher resolution (5-6 µm of axial resolution and 20 µm of transverse resolution), and better visualization. This high-speed scanning makes ocular movements negligible during measurements, which results in a good accuracy and repeatability.\textsuperscript{40,46-47} The Pentacam is a Scheimpflug-base system that, non-invasively determines CCT by acquiring images on the front and back corneal surface. As mentioned above, there are controversy on CCT measurement using the Scheimpflug-base systems compared to USP.\textsuperscript{33-38} The tono-pachymeter CT-1P uses light reflection by the front and backside of the cornea. The reflected light was brought in by the line sensor. The CCT was
measured according to the interval between the front and backside reflection images on the line sensor, so the corneal limits detection may be different than those obtained by ultrasound reflection or the Scheimpflug-base system.\textsuperscript{28,43} Despite the implications derived from the different algorithms that are used for CCT calculation, the differences could result too from the fixation. Pentacam, AS-OCT and CT-1P tono-pachymetry have macular fixation points conditioning by the capture process, while USP is obtained by the clinician choosing where to make the measurement. These phenomena, also might be considered a measure bias in the study and explain in part the differences between non-contact devices and USP. The impact of the tear film on the measurements should also be taken into account.

Although there are various instruments utilizing different principles that can measure CCT showing significant differences, not all are equal in terms of the degree of concordance and interchangeability. Therefore, in this study we have comprehensively analyzed the relationship among the CCT values obtained using USP, Pentacam, AS-OCT and tono-pachymetry systems, and we have also quantified the limit of agreement (LoA) between the CCT measurements with the pairs as plotted against their mean, using Bland–Altman plots. The mean difference between the measurements on the Bland–Altman plot is an estimate of the fixed bias in the measurements, which is the relationship of the difference in the measurements and the mean of the measurements. Our results show that CCT measurements among USP, Pentacam and AS-OCT were comparable and had significant strong positive correlations. Conversely CT-1P tono-pachymetry show lower correlation and agreement when compared between USP, Pentacam or AS-OCT. Several authors demonstrated that CCT measurements performed using the Pentacam have good correlation and agreement with those performed using USP in healthy myopes.\textsuperscript{12,48-50} Similarly, with measures obtained with different AS-OCT devices.\textsuperscript{51-52} According to all of these results, highest agreement was accepted between Pentacam or AS-OCT and USP, hence, many authors assume that
Pentacam or modern AS-OCT can substitute USP in CCT measurement. Meanwhile, there are a few studies that suggest than Scheimpflug-base tono-pahymeters were similar to Pentacam or USP in terms of agreement.\textsuperscript{40-42} However recently Sagdik et al, found a similar agreement between CT-1P and USP which suggest that this tono-pachymeter cannot be interchangeably used with USP or the other non-contact devices because of broad 95% LoA between the pairs in normal eyes.\textsuperscript{43}

As indicated in the methods, reliability value ranges between 0 and 1, with values closer to 1 representing stronger reliability. Historically, Pearson correlation coefficient, paired $t$ test or ANOVA, and Bland-Altman plot have been used to evaluate reliability.\textsuperscript{53-54} However, paired $t$ test or ANOVA and Bland-Altman plot are methods for analyzing agreement, and Pearson correlation coefficient is only a measure of correlation, and hence, separately they are “non-ideal” measures of reliability. However, ICC reflects both degree of correlation and agreement between measurements which indicates reliability. Thus, for a more complete analysis, ICC was assessed. ICC shows an excellent reliability between pairs except for CT-1P against the other three instruments that were found moderate. The relationship between values obtained by AS-OCT compared with the USP showed the highest ICC, while matching values obtained with CT-1P tono-pachymetry and USP had the lowest ICC (Table 2). These relationships, coupled with the differences, confirm that the CCT measurements obtained by CT-1P tono-pachymetry are not interchangeable with those obtained by USP. On the contrary, the lower differences between AS-OCT or Pentacam when compared with USP and their high ICC suggest the possibility of interchanging their values.

There are some limitations to this study. We excluded subjects with severe myopia, astigmatism of more than 1.75 D, irregular astigmatism, refractive surgery, and ocular pathologies, for which any bias between instruments could have clinical implications, and thus, our findings may hold true only for subjects with similar refraction
characteristics. Furthermore, the sample size of this study was relatively small, future studies will need to include larger populations, with different ocular conditions.

In conclusion, our data suggest that the clinician should be aware of significant differences of CCT values when measuring with different devices. Furthermore, in clinical settings where CCT values are critical, we suggest that the CCT results of the CT-1P versus USP and the CT-1P versus Pentacam or CT-1P versus AS-OCT should not be used interchangeably. Given mean differences and range variations in CCT measurements between devices, AS-OCT, Scheimpflug-based system and USP could be interchangeable to measure CCT in healthy subjects. However, in clinical practice, these three modalities should be tested in different pathologic conditions. Although CCT values measured with Pentacam, AS-OCT and USP are closely similar, clinicians should keep in mind that these methods are not simply interchangeable.

References


Table 1. Comparisons among pairs of instruments, average difference, upper and lower 95% confidence limits, statistical significance and Pearson correlation coefficient. Values are in microns. Calculation in SPSS using multivariate analysis of variance (ANOVA) in the general lineal model.

<table>
<thead>
<tr>
<th>Pairwise Comparison</th>
<th>Mean Diff.</th>
<th>95% Confidence Interval</th>
<th>Sig (p-value)*</th>
<th>Correlation Pearson</th>
</tr>
</thead>
<tbody>
<tr>
<td>USP vs Pentacam</td>
<td>8.5±10.2</td>
<td>9.4 to 26.4</td>
<td>&lt;0.001</td>
<td>0.946</td>
</tr>
<tr>
<td>USP vs 3D OCT</td>
<td>13.0±8.4</td>
<td>4.9 to 30.9</td>
<td>&lt;0.001</td>
<td>0.979</td>
</tr>
<tr>
<td>USP vs CT-1P</td>
<td>33.1±33.3</td>
<td>-15.2 to 50.9</td>
<td>&lt;0.001</td>
<td>0.640</td>
</tr>
<tr>
<td>Pentacam vs 3D OCT</td>
<td>4.5±9.5</td>
<td>13.3 to 22.4</td>
<td>0.001</td>
<td>0.951</td>
</tr>
<tr>
<td>Pentacam vs CT-1P</td>
<td>24.6±30.8</td>
<td>-6.7 to 42.5</td>
<td>&lt;0.001</td>
<td>0.700</td>
</tr>
<tr>
<td>3D OCT vs CT-1P</td>
<td>20.0±31.3</td>
<td>-2.1 to 37.9</td>
<td>&lt;0.001</td>
<td>0.687</td>
</tr>
</tbody>
</table>


Table 2. Results of intraclass correlation coefficient (ICC), upper and lower 95% confidence limits and statistical significance. Calculation in SPSS using two-way random model.

<table>
<thead>
<tr>
<th>Pairs-Parameter</th>
<th>ICC</th>
<th>95% Confidence Interval</th>
<th>Sig (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>USP vs Pentacam</td>
<td>0.946</td>
<td>0.807 to 0.968</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>USP vs 3D OCT</td>
<td>0.978</td>
<td>0.863 to 0.987</td>
<td>&lt; 0.001</td>
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<tr>
<td>USP vs CT-1P</td>
<td>0.608</td>
<td>0.303 to 0.759</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Pentacam vs 3D OCT</td>
<td>0.951</td>
<td>0.839 to 0.972</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Pentacam vs CT-1P</td>
<td>0.660</td>
<td>0.375 to 0.790</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>3D OCT vs CT-1P</td>
<td>0.648</td>
<td>0.347 to 0.781</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Figure 1. Scattered plot analysis of CCT showing a significant positive strong correlation between CCT measured by: A) USP and Pentacam (slope=0.946, $R^2=0.895$); B) USP and 3D OCT-2000 (slope=0.979, $R^2=0.957$); C) Pentacam and 3D OCT-2000 (slope=0.951, $R^2=0.905$); D) USP (slope=0.640, $R^2=0.409$); E) Pentacam (slope=0.700, $R^2=0.490$); F) 3D OCT-2000 (slope=0.687, $R^2=0.471$).
Figure 2 Bland-Altman analysis. Mean difference (solid line) and 95% limits of agreement-LoA (dashed line) for CCT. Mean differences were: A) 8.5 μm with 95% LoA from -11.4 to 28.4 μm for the USP/Pentacam pair; B) 13.0 μm with 95% LoA from 0.4 to 25.6 μm for the USP/AS-OCT pair; C) 4.5 μm with 95% LoA from -14.1 to 23.2 μm for the Pentacam/AS-OCT pair; D) 33.1 μm with 95% LoA from -32.2 to 98.3 μm for the USP/CT-1P pair; E) 20.0 μm with 95% LoA from -
41.4 to 81.5 μm for the AS-OCT/CT-1P pair; F) 24.6 μm with 95% LoA from -35.7 to 84.9 μm for the Pentacam/CT-1P pair.