Reproducibility of Cornea Measurements in Anterior Segment OCT Images of Normal Eyes and Eyes with Bullous Keratopathy Analyzed with the Zhongshan Assessment Program

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PURPOSE. To determine the interobserver and intraobserver measurement reproducibility of cornea parameters of both normal eyes and eyes with bullous keratopathy (BK) obtained with the Zhongshan Assessment Program (ZAP) on anterior segment optical coherence tomography (AS-OCT) images.

METHODS. A comparative study was carried out on 24 healthy volunteers and 25 subjects with BK. AS-OCT images were independently analyzed by two examiners. Parameters examined: anterior chamber depth (ACD), central corneal thickness (CCT), posterior corneal curvature (PCC), and posterior corneal arc length (PCAL). Interobserver and intraobserver reproducibility of these parameters was calculated in terms of limits of agreement (mean of differences ± 1.96SD of differences).

RESULTS. In the normal group, both horizontal and vertical ACD were successfully measured in 23 of 24 (96%) images. The mean bias for two measurements by two different observers ranged from 0.003 to 0.117 mm for ACD, PCC, and PCAL measurements and from 0.013 to 2.25 μm for CCT measurements, and there were no differences between the two observers (P > 0.05). Mean bias for two measurements by the same grader ranged from 0.005 to 0.327 mm for ACD, PCC, and PCAL measurements and 1.46 to 2.53 μm for CCT measurements. There was no difference between the two observations (P > 0.05). Similar results were found in the BK group.

CONCLUSIONS. There was high inter- and intraobserver reproducibility for normal and pathologic corneas using the ZAP software. The ZAP software may serve as a new investigatory tool for accurately evaluating the anterior segment and corneal parameters for corneal procedures. (Invest Ophthal Vis Sci. 2011;52:8884–8890) DOI:10.1167/iovs.10-6411

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prised 25 subjects with BK. The study had the approval of the hospital’s Ethics Committee and was conducted according to the tenets of the Declaration of Helsinki. Written informed consent was obtained from study subjects after the nature and intent of the study had been fully explained to them. The exclusion criteria for the normal group were any ocular abnormalities, history of eye disease, prior refractive surgery, and contact lens wear. In the BK group, eyes with mild to moderate corneal clouding affecting visual acuity and requiring DSAEK were included. The authors chose this condition because it is common and mild to moderate corneal clouding will have only a minimal effect on AS-OCT image acquisition (see Figs. 2 and 3 in the following text). This disease is the primary indication in which PCAL will be most useful to measure graft size from the posterior border. The inclusion criteria are all patients with mild to moderate corneal edema affecting visual acuity requiring DSAEK.

All scanning was performed in the undilated left eye in the normal group. In the BK group, scanning was done in the pathologic eye. This was the right eye in 10 subjects and the left eye in the remaining 15 subjects. During scanning, subjects were asked to fix on the external fixation lighting the contralateral eye. The external fixation light was positioned so that the subject was looking straight ahead.

**Scanning**

Performance of the AS-OCT (Visante; Carl Zeiss Meditec, Dublin, CA) has been described previously.10 Study subjects underwent slit-lamp examination, in a sitting position by a single examiner (JC), before imaging. AS-OCT images of the anterior chamber were obtained under dark conditions using the standard anterior segment single-scan protocol. To obtain the best-quality image, the examiner adjusted the saturation and noise and optimized the polarization for each scan during the examination.

Patients were instructed to keep their eyes wide open during scanning and, when necessary, the lids were gently held apart (with care not to exert pressure on the globe) to ensure that the lids did not block the 10-mm-diameter corneal mapping. The operator adjusted the system to position the vertex at the center of the AS-OCT image and to maximize the vertex reflection. All scanning was performed by the same operator (JC). All measurements were taken between 10:00 AM and 4:00 PM (to minimize any effect of diurnal variation on corneal thickness).11–15

In the control group, both vertical and horizontal measurements were taken and compared to validate the software, despite the vertical measurement being harder to obtain. In the BK group, measurements were taken only in the horizontal meridians because having already validated the controls in both meridians, we wanted to validate only the consistency of horizontal scans that were part of the study. Vertical scans were more difficult to obtain, for AS-OCT imaging and for scleral spur identification, due to eyelid blocking, and thus not used in the diseased group.

**Image Processing and Analysis**

The AS-OCT scans were processed using in-built dewarping software that adjusted for image distortion resulting from corneal optical properties. Scleral spur location was the only observer input required during image assessment. This was defined by a prominent inner extension of the sclera at its thickest part,14–16 and often appeared as an inward protrusion or change in the curvature of the inner surface of the angle wall.

The Zhongshan Assessment Program (ZAP, Guangzhou, China) software automatically extracted the 300 × 600 8bit grayscale intensities from 0 to 255 image portion of the output file and performed noise and contrast conditioning. A binary copy of the image was then produced where pixels were either 1’s (tissue) or 0’s (open space) depending on whether they were brighter or darker than a calculated threshold value. Algorithms defined the borders of the corneal epithelium and endothelium and the anterior surface of the iris. The algorithms used basic edge arguments (5 consecutive 0’s above, and 5 consecutive 1’s below indicated an anterior surface point) to describe the borders. The corneal border data were fitted with polynomial curves and a line-smoothing algorithm, explicitly defined by the edge finding algorithms, used the derivative data to repair step-like portions of the border. The radius (in mm) of the curve on the central one third of the posterior cornea was calculated as PCC.

The parameters obtained from image analysis included PCC (in mm), CCT (in μm), anterior chamber depth (ACD, in mm), and PCAL (in mm). PCC is defined as the radius of curvature of the posterior surface of the cornea. CCT was measured from the anterior to the posterior surface of the cornea, taken at the center of the AS-OCT image. ACD was measured from the posterior surface of the cornea to the anterior surface of the lens, also taken at the center of the AS-OCT image. PCAL is a novel parameter, defined as the arc distance between two scleral spurs along the corneal endothelium (Fig. 1).

**Repeatability of Image Analysis**

Two ophthalmologists (JC and EH) independently carried out the image analysis for the full set of images of the first group (normal subjects) using the ZAP software. One week later, one of the examiners (JC) repeated the analysis for the same set of images and she was masked to the results of initial analysis.

For the BK group, two ophthalmologists (JC and LY) carried out the image analysis for the full set of images of the second group using the ZAP software. One week later, one of the examiners (JC) repeated the analysis for the same set of images and again she was masked to the results of initial analysis. Examples of images from the BK group are shown in Figures 2 and 3.

**Statistical Methods**

Bland Altman analysis was performed to analyze inter- and intraobserver agreement (MedCalc, Version 9.6.4.0; MedCalc Software, Mari-

**Figure 1.** An AS-OCT image analyzed by ZAP software.

**Figure 2.** AS-OCT image of bullous keratopathy showing good penetration and minimal effect of corneal edema on scan.
akerke, Belgium). Interobserver and intraobserver reproducibility of the above parameters was calculated in terms of limits of agreement (LOA; mean of differences ± 1.96SD of differences). Paired t-tests were used for the differences between observer measurements. Statistical analysis was performed with data analysis/spreadsheet software (Microsoft Excel; Microsoft Corp., Redmond, WA).

**RESULTS**

### Analysis in the Normal Group

The normal group of study participants consisted of 24 volunteers (10 males and 14 females), ranging in age from 21 to 55 years (mean age, 30.48 years). The ethnicity of normal subjects was distributed as follows: 12 Chinese, 7 Indian, 3 Malay, and 2 Burmese.

**Intraobserver Reproducibility.** In both horizontal and vertical meridians, the mean of differences (bias) between two repeated measurements by the same grader was small, and LOA values were correspondingly small. In the horizontal meridian, intraobserver bias was 0.196 mm for PCAL \((P = 0.189)\), 0.005 mm for ACD \((P = 0.941)\), and 0.055 mm for PCC \((P = 0.613)\) measurements, respectively, and was 2.533 \(\mu m\) for CCT \((P = 0.788)\) measurements. In the vertical meridian, intraobserver bias was 0.327 mm for PCAL \((P = 0.082)\), 0.015 mm for ACD \((P = 0.649)\), and 0.006 mm for PCC \((P = 0.946)\) measurements, and was 1.463 \(\mu m\) for CCT \((P = 0.874)\) measurements (Table 1 and Fig. 3).

**Interobserver Reproducibility.** Comparing parameter for parameter, the mean of differences between two graders was lower for all parameters except horizontal ACD and horizontal PCC. However, the LOA values in the interobserver agreement were similar to those in the intraobserver agreement for both horizontal and vertical scans. Interobserver bias in horizontal measurements was 0.025 mm for PCAL \((P = 0.857)\), 0.009 mm for ACD \((P = 0.901)\), 0.086 mm for PCC \((P = 0.446)\) measurements, and was 0.013 \(\mu m\) for CCT \((P = 0.999)\) measurements. Interobserver bias in vertical measurements was 0.117 mm for PCAL \((P = 0.498)\), 0.003 mm for ACD \((P = 0.967)\), 0.017 mm for PCC \((P = 0.843)\) measurements, and was 2.250 \(\mu m\) for CCT \((P = 0.804)\) measurements.

### Analysis in the BK Group

The BK study group consisted of 25 patients with BK of varying causes. There were 9 males and 16 females, ranging in age from 56 to 79 years. The ethnicity of subjects with BK was distributed as follows: 17 Chinese, 5 Indonesian, 2 Vietnamese, and 1 Eurasian. All parameters were measured in the horizontal meridian only.

**Intraobserver Reproducibility.** As with the normal group, the mean of differences and limits of agreement for repeated measurements by the same grader were small. Agree-
### Table 1. Interobserver and Intraobserver Variability of Parameters of Normal Corneas Analyzed with ZAP Software

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean (SD)</th>
<th>Mean (SD)</th>
<th>Mean Bias (95% CI)</th>
<th>Limits of Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observer 1</td>
<td>Observer 2</td>
<td>(Two-sided)</td>
<td>Lower Limit</td>
</tr>
<tr>
<td></td>
<td>First Analysis</td>
<td>Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Second Analysis</td>
<td>Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interobserver variability</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horizontal PCAL</td>
<td>12.928 (0.447)</td>
<td>12.903 (0.483)</td>
<td>0.857</td>
<td>−0.025 (−0.145 to 0.095)</td>
</tr>
<tr>
<td>Horizontal CCT</td>
<td>573.000 (33.240)</td>
<td>573.013 (32.659)</td>
<td>0.999</td>
<td>0.013 (−2.865 to 2.890)</td>
</tr>
<tr>
<td>Horizontal ACD</td>
<td>3.161 (0.240)</td>
<td>3.162 (0.238)</td>
<td>0.901</td>
<td>0.009 (−0.009 to 0.026)</td>
</tr>
<tr>
<td>Horizontal PCC</td>
<td>6.440 (0.389)</td>
<td>6.527 (0.388)</td>
<td>0.446</td>
<td>0.086 (−0.036 to 0.209)</td>
</tr>
<tr>
<td>Vertical PCAL</td>
<td>13.298 (0.599)</td>
<td>13.182 (0.584)</td>
<td>0.498</td>
<td>−0.117 (−0.212 to −0.021)</td>
</tr>
<tr>
<td>Vertical CCT</td>
<td>569.480 (31.331)</td>
<td>569.729 (31.068)</td>
<td>0.804</td>
<td>2.250 (−0.262 to 4.762)</td>
</tr>
<tr>
<td>Vertical ACD</td>
<td>3.149 (0.236)</td>
<td>3.146 (0.241)</td>
<td>0.967</td>
<td>−0.003 (−0.006 to 0.000)</td>
</tr>
<tr>
<td>Vertical PCC</td>
<td>6.410 (0.277)</td>
<td>6.428 (0.317)</td>
<td>0.845</td>
<td>0.017 (−0.038 to 0.072)</td>
</tr>
</tbody>
</table>

CI, confidence interval.
ment for intraobserver measurements was equally strong for all parameters measured. The bias was 0.06 mm for PCAL ($P = 0.104$), 0.037 mm for ACD ($P = 0.275$), and 0.059 mm for PCC ($P = 0.065$) measurements, and was 0.371 $\mu$m for CCT ($P = 0.767$) measurements (Table 2).

**Interobserver Reproducibility.** When horizontal measurements were performed by two graders, the mean of differences was smaller than that of repeated horizontal measurements by the same grader. The interobserver bias was 0.0017 mm for PCAL ($P = 0.761$), 0.00046 mm for ACD ($P = 0.741$), and 0.043 mm for PCC ($P = 0.311$) measurements, and was 1.57 $\mu$m for CCT ($P = 0.315$) measurements. The limits of agreement for measurements by two graders was correspondingly small. Agreement for interobserver measurements was equally strong for all parameters measured (Table 2 and Fig. 4).

**DISCUSSION**

In this study, we found that the ZAP software was a highly reproducible tool for quantitative analysis of corneal parameters measured on AS-OCT. Mean of differences between measurements by the same grader was found to be small for parameters in both the normal and the BK groups. Means of differences between measurements by different observers were also found to be small in both groups, and limits of agreement were high for both interobserver and intraobserver variation. Interestingly, $P$ values were marginally significant for two parameters measured by the same observer. These parameters were vertical PCAL in the normal group ($P = 0.082$) and horizontal PCC in the BK group ($P = 0.065$). These same parameters had high $P$ values for measurements by different observers. There does not appear to be a reason for the low $P$ values; moreover, all $P$ values were found to be $>0.05$. Because the reproducibility of AS-OCT has been previously documented, these observations validate the reproducibility and thus the clinical utility of the ZAP software, given that it demonstrates that differences between users of the software tend to be small; thus valid comparisons between analyses by different users can be made. In addition, this study found that the ZAP software showed the repeatability of measurements in both normal and diseased corneas, thus validating its use in a clinical setting to diagnose and follow-up cornea pathology.

The cornea parameters measured in this study included the anterior chamber depth (ACD), posterior cornea curvature (PCC), central cornea thickness (CCT), and posterior corneal arc length (PCAL). The PCAL is a novel cornea parameter that has not previously been accurately and reproducibly measured, and to our knowledge, the ZAP software is the only image processing software able to do so. Although the other three parameters (ACD, PCC, and CCT) can be manually measured on AS-OCT scans using the built-in measurement tool, image processing using the ZAP software uses algorithms to identify the borders of the cornea endothelium, epithelium, and anterior surface of the iris. This avoids potential inaccuracies that can arise from manual placement of the AS-OCT measurement tool on the AS-OCT image, which may be done in an arbitrary fashion. In addition the software gives new values not able to be calculated with current “in-built” software.

The ability to reproducibly measure cornea parameters has a myriad of clinical and research applications. In particular, the advent of lamellar and refractive cornea procedures necessitates accurate measurement of the CCT and PCAL. The PCAL is a novel parameter defined as the posterior corneal border distance between scleral spurs. This gives a good estimation of the limits of the diameter of the corneal endothelium. Its potential utility is particularly relevant to endothelial keratoplasty (EK) procedures (such as DSAEK and Descemet’s mem-

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**Table 2.** Interobserver and Intraobserver Variability of Horizontal Parameters of Corneas with Bullous Keratopathy Analyzed with ZAP Software

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Observer 1</th>
<th>Mean (SD)</th>
<th>First Analysis</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
<th>P</th>
<th>Mean Bias (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interobserver</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACD</td>
<td>4.19 (1.95)</td>
<td>3.16 (2.03)</td>
<td>0.741</td>
<td>0.00046</td>
<td>0.003 to 0.0024</td>
<td>0.371</td>
<td>0.059 (0.031 to 0.104)</td>
</tr>
<tr>
<td>PCC</td>
<td>6.86 (0.73)</td>
<td>6.92 (0.73)</td>
<td>0.275</td>
<td>0.037</td>
<td>0.02 to 0.009</td>
<td>0.043</td>
<td>1.57 (0.29 to 1.86)</td>
</tr>
<tr>
<td>CCT</td>
<td>774.64 (130)</td>
<td>775.01 (127.89)</td>
<td>0.767</td>
<td>0.064</td>
<td>0.123 to 0.013</td>
<td>0.104</td>
<td>0.059 (0.047 to 0.071)</td>
</tr>
<tr>
<td>PCAL</td>
<td>12.87 (0.64)</td>
<td>12.92 (0.65)</td>
<td>0.104</td>
<td>0.066</td>
<td>-0.134 to 0.015</td>
<td>0.341</td>
<td>0.165 (0.115 to 0.215)</td>
</tr>
</tbody>
</table>

**CI:** confidence interval.
brane endothelial keratoplasty), where accurate preoperative measurement of the PCAL in a recipient can guide an appropriate choice of donor graft diameter. Currently, most surgeons “guesstimate” the appropriate EK size graft from measurements of the anterior corneal surface, similar to what is conventionally done for penetrating keratoplasty (PK). The advantage of an EK procedure is that the surgeon can transplant much larger grafts, and thus more endothelium than that for conventional PK. In our center, the mean size of the EK graft is 8.75 mm (range, 7–10 mm), mode 9 mm, whereas that for PK is 8 mm (range, 6.5–11.5 mm), mode 7.5–7.9 mm. The additional 1-mm increase in the graft area adds a 26% increase in the amount of endothelial cells transplanted. Thus, optimal graft sizing can have a direct impact on the amount of endothelial cells transplanted. The choice for horizontal scans was used because the horizontal meridian is largest and thus most appropriate for DSAEK cases.

AS-OCT can be used to image patients with EK postoperatively, and recent further modification of the ZAP software can be used to calculate the diameter of the implanted endothelial graft, providing useful clinical parameters for subsequent follow-up of these patients. Further work is under way to validate the DSAEK graft measurements from AS-OCT images using a modification of the ZAP software in a comparison with intraoperative graft trephine diameter.

There are a number of other anterior segment procedures for which the ZAP software has potential utility, both in preoperative assessment and in postoperative follow-up. For example, in anterior chamber phakic IOL implantation, measurement of the PCAL and ACD allows the surgeon to accurately select an appropriate size implant preoperatively. Postoperative measurement of these parameters is useful in determining if the implant has been placed in an optimal position; subsequent follow-up measurements can determine whether the implant shifts or subluxes with time. The other strength of this study is the high limits of agreement between observers and those within one observer, suggesting the high repeatability of the software.

The ZAP software has some limitations. First, image processing was dependent on the manual identification of the scleral spur as a measurement reference point. The difficulty of this practice has been well recognized. In a cross-sectional study of 502 eyes, Sakata and colleagues found the detectability of the scleral spur on AS-OCT images to be 72% overall, and was less detectable on AS-OCT images obtained in the superior (64%) and inferior (67%) quadrants. To overcome this limitation, the ZAP software has an in-built magnification window that can be moved to any position on the scan. We found the placement of this window over the scleral spurs to be highly useful in determining their exact position. In addition, during vertical scan acquisition, the subject’s eyelids were gently held apart to ensure that the lids did not obscure the scleral spurs. This increased the detectability of the scleral spur in our study, and we were able to detect the scleral spur in all
AS-OCT images analyzed. This is particularly important if one is planning on using this software to estimate the graft size since the major limitation will be the vertical graft size.

The ZAP software is also limited in that it is unable to process high-resolution spectral-domain (SD) OCT images. This is due to the limited 6-mm scan width of the SD-OCT, which is unable to image more than one angle concurrently in one image. Thus, the ZAP software is able to process only time-domain OCT images, which have a lower resolution but a larger scan width than can encompass both angles in the same image.

Finally, the limitation of this study lies in the small sample size, and that analysis was done in a variety of bullous keratopathies that were not stratified. In conclusion, the Zhongshan Assessment Program offers accurate measurement of corneal parameters from AS-OCT images with good inter- and intraobserver reproducibility, for scans in both the horizontal and vertical meridians. The program is relatively easy to run, with manual identification of the sclera spur as the only observer input required. The data obtained from the scans allow detailed quantitative analysis of the anterior segment, which is useful for the preoperative planning of many anterior segment procedures.

References