The effects of corneal parameters on the assessment of endothelial cell density in the elderly eye

A Müller, J P Craig, C N Grupcheva, C N J McGhee

Background: The possible impact of corneal thickness, curvature, and size on the measurement of endothelial cell density (ECD) has largely been ignored in the normal eye. The aim of this study was to investigate the possible impact of the main corneal parameters on the analysis of ECD values at the central, superior, and temporal parts of the corneal surface.

Methods: All 75 participants (52 females, 23 males) were assessed as part of a pre-cataract surgery investigation. The mean age was 75.7 (SD 10.9) years. Confocal microscopy was used to measure ECD and the percentage of six sided cells at the central, superior, and temporal parts of the cornea. The Orbscan II topography system was used to measure corneal thickness, topography, and horizontal corneal diameter.

Results: The mean central ECD measured was 2488 (SD 301) cells/mm², compared with 2525 (SD 505) cells/mm² in the temporal cornea and 2639 (SD 398) cells/mm² in the superior cornea. The regional differences in ECD were not significant (p>0.14). The central ECD was significantly correlated to the central (mean 0.593 (SD 0.039) mm, p = 0.021) as well as the temporal (0.628 (SD 0.039) mm, p<0.001) and the superior corneal thickness (SD 0.644 (SD 0.048) mm, p = 0.018). The mean corneal curvature at the centre (7.7 (SD 0.34) mm, p = 0.002) as well as 3 and 5 mm from the apex was significantly related to ECD (p=0.008 and p=0.009, respectively).

Conclusions: The study suggests that in an older population, lower ECD values would be expected in thinner and/or steeper corneas.

During the early years of life, endothelial cell density (ECD) undergoes a more rapid decline compared with that in later life. Although the main cause of the rapid decline in ECD has been accounted for by enlargement of the posterior corneal surface, the possible impact of corneal size, shape, and thickness on changes in ECD throughout life has largely been ignored. While a large horizontal corneal diameter was shown to correlate with a lower ECD count during early teenage years, ECD in young adults was reported to be lower in longer and flatter myopic eyes. The corneal parameter that has most commonly been assessed for its effect on ECD is corneal thickness. Although the true role of corneal thickness in the assessment of ECD in the normal eye is ambiguous, the correlation between endothelial cell dysfunction and increased corneal thickness is well established. Causes for increased corneal thickness include various diseases of the anterior segment of the eye, contact lens wear, and intraocular surgery. There is also a sustained interest in the potential of corneal thickness to act as a indicator for numerous systemic and ocular diseases, including diabetic retinopathy, glaucoma, and uveitis. Besides predicting corneal disease or low endothelial cell counts, corneal thickness per se is also used to evaluate endothelial function in the normal cornea. However, few studies have investigated a possible correlation between corneal thickness and endothelial cell density in the normal eye.

Although corneal thickness is generally independent of the other morphometric parameters of the normal human eye and does not change significantly during life, there are substantial differences in individual corneal thickness values. Whether such differences somehow affect the measurement of cell areas and therefore the analysis of ECD, remains unknown. Contradictory outcomes of studies that have reported the possible correlation between corneal thickness and ECD in the past could be multifactorial. The primary limitation of previous investigations is that conclusions have been drawn based on the assessment of the central corneal endothelium, it is limited in its ability to evaluate the far periphery of the cornea. Correlation of these two parameters—corneal thickness and endothelial cell density—may be beneficial for morphological studies of the normal human cornea. Large interstudy variations may also partly be explained by substantial differences in the methodology and technology used for ECD estimation.

In the present study, corneal thickness, as measured by slit scanning topography and ECD values were assessed at the corneal centre as well as in the temporal and superior corneal periphery. Using in vivo confocal microscopy for the evaluation and analysis of ECD, the peripheral endothelial layer could be assessed. Although the specular microscope, as mainly used in reported studies, is useful for imaging the central corneal endothelium, it is limited in its ability to evaluate the far periphery of the cornea. Most studies, comparing central and peripheral ECD, describe areas that were more than 1 mm from the limbus. In the present study, however, the confocal microscope allowed imaging of the far peripheral endothelium.

The main aim of this study was to investigate the possible impact of corneal thickness, anterior and posterior corneal shape, corneal diameter, axial length, and refractive error of the eye on the analysis of ECD values across the corneal surface.

METHODS

Subjects
Seventy five participants (52 females, 23 males) were assessed as part of a pre-cataract surgery investigation.

Abbreviations: CCT, central corneal thickness; ECD, endothelial cell density
mean age was 75.7 (SD 10.9) years, ranging between 48 and 91 years. Of all participants, the majority described themselves to be of European origin (50), followed by Maori (10), Pacific Islanders (6), Asians (4), and others (6). Consent was given by all participants and ethical approval for this study was obtained from the local Auckland Ethics Committee.

MEASUREMENTS

ECD assessment
Prior to confocal microscopy, one drop of topical anaesthetic (Ophthalmic proparacaine hydrochloride 5 mg/ml, Allergan Australia Pty Ltd, Gordon, NSW, Australia) was instilled in the eye to be examined. One pea sized drop of immersion gel (Viscotears, Allergan Australia Pty Ltd) was applied to the tip of the objective lens of the confocal microscope (ConfoScan 2.0, NIDEK Technologies, Greensboro, NC, USA). The microscope was brought into position using a joystick and the lens was aligned with the eye. With the drop of gel in contact with the surface of the eye, scan images of the central cornea were captured. The patient was then asked to fixate on set targets placed 2.5 mm off the central gaze and approximately 7 cm from the eye, so that the temporal and superior parts of the cornea (approximately 1 mm from the limbus) could be scanned.

Approximately 300 images were obtained per scan. Based on the best visibility of endothelial cells, three representative frames from each scan were chosen for analysis. The maximum examination time was 10 minutes in total. All captured images were analysed using the NAVIS (NIDEK Technologies). The chosen frame size, or region of interest (ROI), was 0.035 mm². Using manual assisted cell counts, as performed by the software, the number of clearly visible cells were counted. The mean values for endothelial cell density, the percentage of six sided cells within each of the three frames were recorded and averages for each cornea were calculated.

Corneal parameters
The Orbscan II slit scanning corneal topography system (Orbscan, Bausch and Lomb, Salt Lake City, UT, USA) was used to measure corneal thickness and horizontal corneal diameter, and assess corneal topography. Before measurement, the subject’s head was aligned with the instrument and a head strap was placed around the back of the head. The subject was advised to keep both eyes open and fixate on the target. By viewing the live image of the eye on the monitor, the examiner aligned the two fixation markers reflected by the instrument on the corneal surface before performing the scan. Three scans were performed per cornea and the mean values for the central, temporal, and superior corneal thickness, the horizontal diameter, the spherical equivalent (least square method of determining best fit sphere), and eccentricity of the anterior and posterior surface were recorded. The instrument is designed in a way that peripheral corneal thickness is measured 3 mm from the corneal centre, within a circle of 2 mm. Axial length was measured with an A-scan ultrasond (Tomey AL-2000, Tomey, Erlangen, Germany) and an autorefractor (Topcon KR-8100 Topcon America, Paramus, NJ, USA) was used for refractive error assessment. For each eye, a minimum of 10 axial length recordings were made and the mean calculated. Three measures were taken for each of the later two investigations and the mean value used for statistical analysis.

Visual acuity
Visual acuity was measured using a single new, calibrated, 4 m illuminated LogMAR chart (University of Otago, NZ).

Statistical analysis
The Kolmogorov-Smirnov test was used to assess normality and the level of significance was set at p<0.05. According to whether or not the distributions of data sets were normal, the means of variables between different groups were compared by Student’s t tests or a Mann-Whitney U test, respectively. Again, the level of significance was set at p<0.05. The Pearson coefficient of correlation was used to test correlation between parameters. If the Pearson correlation analysis revealed any parameters to be correlated, the significance of these variables was further tested by multiple regression analysis. The value of significance of the slopes was defined (p value) as well as the value for the correlation coefficient (r value). Mean (standard deviation, SD) values are described.

RESULTS

Central endothelial cell density
The ECD characteristics for the central, temporal and superior cornea are shown in table 1. The analysis of each cornea included between 77 and 131 clearly visible cells. There was no significant sex related difference in central ECD (mean ECD in females 2494 (SD 300) cells/mm², mean ECD in males 2472 (SD 303) cells/mm², p = 0.76) and the values were normally distributed (p=0.15).

Peripheral endothelial cell density
Both mean and median ECD, assessed for the temporal and superior part of the cornea, are shown in table 1. The mean temporal ECD value was slightly higher than the central ECD value (by 2%). Compared with the average central ECD value, superior ECD was 4% higher. Temporal and superior ECD values were slightly skewed (p = 0.01 and p = 0.041, respectively) and the differences in mean ECD between central, temporal, and superior cornea were not significant (p>0.05). There were also few, if any, differences with respect to sex in all three areas assessed and none could be shown to be significant (p>0.7).

Percentage of six sided cells
Differences in the type of endothelial cells are commonly investigated by assessing the proportion of cells that are six sided (‘‘percentage of hexagonality’’). Values of six sided cells, measured centrally for the 75 corneas examined, are shown in table 1. Values were normally distributed (p>0.05) with a small sex difference (males 48.1 (SD 6.5) %, females 46.5 (SD 5.9) %), which was not significant (p = 0.31). Comparison of the percentage of six sided cells in the central cornea between subjects of European origin and of Maori origin revealed identical mean values (European 47.0 (SD 6.6) %, Maori 47.0 (SD 5.2) %). The mean numbers of six sided cells at the central part (p = 0.97). Values for each site were normally distributed (p>0.15). The mean thickness of the temporal cornea was 23.6% higher than the central corneal thickness (0.628 (SD 0.039) mm, range 0.553–0.729 mm, median 0.627 mm) but slightly lower than the superior cornea (0.644 (SD 0.048) mm, range 0.434 to 0.754 mm, median 0.635 mm). Superior thickness was 26.7% higher than CCT, table 1. All regional differences in corneal thickness were significant
The effects of corneal curvature

The mean anterior curvature (as measured with the Orbscan II instrument) for all corneas assessed was 0.317 (SD 0.363) (median 0.370), indicating that the mean anterior cornea assessed followed the shape of a prolate ellipse (flattening peripherally). The mean overall anterior corneal curvature was 7.77 (SD 0.34) mm (median 7.78 mm), ranging from 7.14 to 8.83 mm and values were skewed (p<0.01). Statistical analysis showed the overall anterior corneal curvature (equivalent of spherical radius of curvature) to be significantly related to mean central ECD (Pearson’s \( r = 0.36 \), \( p = 0.002 \); multiple regression \( p = 0.002 \), (fig 4).

The same positive correlation was found when central ECD was compared with the anterior curvature at 3 mm (\( r = -0.311 \), \( p = 0.009 \)) and at 5 mm from the corneal apex (\( r = -0.317 \), \( p = 0.008 \)). Results suggest that lower ECD values would be expected in steeper corneas.

The mean posterior corneal curvature (equivalent of spherical radius of curvature) was 6.68 (SD 0.36) mm (range 5.9 to 7.57 mm, median 6.64 mm). The distribution of values appeared normal (\( p = 0.15 \)). Again, the mean eccentricity of 0.350 (median 0.385) indicated peripheral flattening of the posterior surface. Although there was a clear trend for the average posterior curvature to be positively correlated to central ECD, this trend was not significant (\( r = 0.21 \), \( p = 0.08 \)). The small differences with respect to sex in average anterior and posterior curvature were not significant (\( p = 0.41 \) and \( p = 0.09 \), respectively).

Axial length of the eye

The mean axial length of the eyes studied was 23.16 (SD 0.93) mm (median 22.99, range 21.71–27.12 mm) and values were normally distributed (\( p = 0.15 \)). There was a small, but not statistically significant, difference between male subjects

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### Table 1: Measured mean values for corneal thickness, ECD, cell area, and percentage of six sided cells at the central, temporal, and superior cornea

<table>
<thead>
<tr>
<th></th>
<th>Central cornea</th>
<th>Temporal cornea</th>
<th>Superior cornea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>CT (mm)</td>
<td>0.593 (0.039)</td>
<td>0.594</td>
<td>0.508–0.693</td>
</tr>
<tr>
<td>ECD (cells/mm²)</td>
<td>2488 (229)</td>
<td>2466</td>
<td>1865–3386</td>
</tr>
<tr>
<td>Cell area (µm²)</td>
<td>409 (49)</td>
<td>410</td>
<td>296–536</td>
</tr>
<tr>
<td>Six sided cells (%)</td>
<td>47.0 (6.1)</td>
<td>47.0</td>
<td>32.0–60.0</td>
</tr>
</tbody>
</table>

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\[ y = 3.3273x + 403.61 \]

\[ y = 2.1237x + 1235.9 \]

\[ y = 2.2422x + 1051.7 \]
(mean 23.33 (SD 1.21) mm) and female subjects (23.15 (0.88) mm). When axial length values were compared with central ECD values, a negative trend was evident (fig 5). The trend suggested that eyes with longer axial length values are associated with a lower central ECD. This trend was statistically significant (Pearson’s $r = -0.235$, $p = 0.04$, multiple regression $p = 0.038$).

**Horizontal corneal diameter**

Comparison revealed central as well as peripheral ECD values to be independent of mean horizontal corneal diameter values (mean 11.78 (SD 0.66) mm, median 11.7 mm, range 10–13.6 mm), indicating that corneal diameter, at least within the age range studied, is not an indicator of cell density. Mean values were normally distributed ($p > 0.15$) and were slightly higher in males (11.9 (0.4) mm) compared with females (11.7 (0.7) mm); however, this difference was not significant ($p = 0.15$).

**Refractive error**

The mean spherical equivalent of the refractive error for all subjects included was $-0.68$ (SD 2.78) D (median 0.0 D, range $-9.13$ to $+4.0$ D). Comparison with axial length values confirmed a trend in which more myopic eyes were longer ($r = -0.323$, $p = 0.005$). Refractive error did not correlate with ECD ($r = 0.078$, $p = 0.74$).

**Visual acuity**

The mean VA (LogMAR) was 0.62 (SD 0.27). The median VA was 0.6 and values reached between 0.2–1.3.

**DISCUSSION**

Although the swelling response of the cornea to any alterations in endothelial barrier and pump functions is well established, there is a lack of information about the possible role of differences in corneal thickness or shape in the assessment of ECD values in the normal eye. Whereas the current study suggests that central ECD values are correlated to the thickness of the central, temporal, and superior cornea within an elderly population, existing studies show substantial variation in outcome. Most studies have reported no correlation between CCT and ECD in the older eye, supporting the theory that corneal thickness is merely an independent parameter of the normal cornea.

Others have suggested the existence of such a correlation in children. In table 2, details on reports assessing the corneal thickness and ECD are summarised. The contradictory reports may be explained by lack of consideration of possible regional differences in ECD distributions and substantial differences in corneal thickness or corneal curvature values across the cornea. In order to understand the potential effect of any changes in corneal parameters on the endothelial cell layer, or its appearance, exact knowledge of the overall cell distribution is needed.

Measurement of the peripheral ECD in vivo is still uncommon and reported values show large variation. Studies have applied either direct microscopic examination of the endothelia of cadaver eyes or, most commonly, used specular microscopy for ECD assessment. Central ECD values in eyes with cataracts, within similar age ranges as the current study, have previously been reported in various studies and the mean values ranged between 2324 and 3093 cells/mm$^2$. The mean value for ECD measured in the current study (2488 cells/mm$^2$) appears to be in agreement with the previously reported values. While ECD values in the literature have most commonly been assessed at the corneal centre only, the present study indicates central ECD values to be 2% lower than the temporal and 4% lower than the superior cornea. There are a number of reports in the literature supporting such a trend of higher cell densities in the peripheral cornea. Sturrock et al, using a specular microscope, reported central ECD values to be 2.8% below nasal ECD and 3.6% below temporal ECD values. Assessing ECD in cadaver eyes, Daus et al reported the maximal peripheral density to be on average 1374 cells/mm$^2$ higher then at the corneal centre. Schimmelpfennig, found ECD values at the far corneal periphery of cadaver eyes to be as much as 30% higher. Some studies have not found any significant regional differences in ECD across the cornea; however, others have reported peripheral ECD values to be lower compared with central ECD values or have suggested that there is a vertical gradient in cell density.

Besides any true regional differences in ECD distribution, there could be some optical, and therefore artefactual, explanation for a correlation between corneal thickness and ECD. The impact of increased peripheral corneal thickness on the magnification of the microscope photographing the endothelial layer was described almost 30 years ago. In the present study, higher ECD values were measured in the flatter periphery of the elliptical cornea. The increased peripheral corneal thickness is compensated by a steeper posterior corneal curvature. When the endothelium is scanned by the confocal microscope, images are taken from a static position, ignoring posterior corneal curvature changes. It could therefore be argued that the appearance of the highly magnified endothelial cells in the corneal periphery is distorted by the steeper angle under which it is photographed. Such a distortion could cause the area of the cells to appear reduced and therefore artificially increase cell density measures in the peripheral cornea.

The present study suggests that lower ECD values would possibly be expected in longer eyes. The same trend has

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**Figure 4** Relation between the measured anterior spherical corneal radius and central endothelial cell density before cataract surgery.

**Figure 5** Relation between measured axial length and central endothelial cell density before cataract surgery.
The effects of corneal parameters

The effects of corneal parameters assessed. The Orbscan instrument allows measures of corneal 

CCT value in the present study is just within these limits 0.535 mm, that is, 0.473–0.597 mm. Although the mean 

adults would be expected to be within SD 11.6% of 

the enlarged surface. This, in return, reduces ECD. However, 

authors have suggested the elongation of the axial length 

small. The percentage of six sided cells appears independent 

cell density and hexagonality, no such differences were found 

significant differences between ethnic groups in endothelial 

surface of the cornea. Whereas Snellingen 

literature. Assessing eyes with cataracts within similar, older 

age groups, studies have reported mean values for six sided 

cells between 51% and 73%. However, in a recent study by 

Snellingen et al., the mean percentage of six sided cells 

measured in 1235 eyes with cataracts, using a semi-

automatic analysis technique, was even lower than in the 

present study (40%). The comparison of cell shapes in the 

central, temporal, and superior part of the cornea revealed no 

significant differences. The minor regional differences in 

values indicate that the different types of cells (based of the 

number of cell sides) are evenly distributed across the inner 

surface of the cornea. Whereas Snellingen et al have reported 

significant differences between ethnic groups in endothelial 

cell density and hexagonality, no such differences were found 

in the present study, comparing subjects of white and Maori 

origin. However, it must be noted that the sample size is 

small. The percentage of six sided cells appears independent 

of any corneal parameters assessed.

In conclusion, the current study suggests that, at least 

within the age range studied, lower endothelial cell density 

values would be expected in thinner and/or steeper corneas. The question of whether these are genuinely related or 

cased by optical artifacts remains unanswered. Based on a 

meta-analysis by Doughty and Zaman, normal CCT in white 

adults would be expected to be within SD 11.6% of 

0.535 mm, that is, 0.473–0.597 mm. Although the mean 

CCT value in the present study is just within these limits (0.593 mm), it could be argued that this is towards the 

higher end of what would be expected for the age group 

assessed. The Orbscan instrument allows measures of corneal 

thickness and curvature at any point on the corneal surface 

and its accuracy, precision, and repeatability have been 

recently been reported by Chang et al. In their study, the 

authors have suggested the elongation of the axial length 

leads to an increase in endothelial surface area. Because of a 
lack of mitotic activity of the corneal endothelium after birth, 

spreading of the endothelial cells is expected in order to cover 

the enlarged surface. This, in return, reduces ECD. However, 

the true optical effects caused by differences in thickness and 

shape of the anterior and posterior corneal surfaces are as yet 

insufficiently assessed. Detailed modelling studies are 

needed, determining the degree to which corneal parameters 

potentially affect the measurement of endothelial cell area, as 

it appears through the specular or confocal microscope.

Although ECD is most commonly used to describe the state 

of health of the corneal endothelium, a high percentage of six 

c sided cells is also seen as an indicator of a healthy corneal 

endothelium. The mean percentage of six sided cells measured in the present study was 47.0 (SD 6.1), which is 

less than most mean values previously reported in the 

literature. Assessing eyes with cataracts within similar, older 

age groups, studies have reported mean values for six sided 
cells between 51% and 73%. However, in a recent study by 

Snellingen et al., the mean percentage of six sided cells 

measured in 1235 eyes with cataracts, using a semi-

automatic analysis technique, was even lower than in the 
present study (40%). The comparison of cell shapes in the 
central, temporal, and superior part of the cornea revealed no 

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number of cell sides) are evenly distributed across the inner surface of the cornea. Whereas Snellingen et al have reported 

significant differences between ethnic groups in endothelial cell density and hexagonality, no such differences were found 
in the present study, comparing subjects of white and Maori 

origin. However, it must be noted that the sample size is small. The percentage of six sided cells appears independent of any corneal parameters assessed.

In conclusion, the current study suggests that, at least within the age range studied, lower endothelial cell density values would be expected in thinner and/or steeper corneas. The question of whether these are genuinely related or caused by optical artifacts remains unanswered. Based on a meta-analysis by Doughty and Zaman, normal CCT in white adults would be expected to be within SD 11.6% of 0.535 mm, that is, 0.473–0.597 mm. Although the mean CCT value in the present study is just within these limits (0.593 mm), it could be argued that this is towards the higher end of what would be expected for the age group assessed. The Orbscan instrument allows measures of corneal thickness and curvature at any point on the corneal surface and its accuracy, precision, and repeatability have been reported to be acceptable for its use in research. However, it is recognised that the Orbscan overestimates corneal thickness compared with the more commonly used technique of ultrasonic pachymetry. The reported difference between the two methods varies from 20 μm to 28 μm, or approximately 5%. One possible explanation is that the Orbscan, due to its non-contact type of measurement, may include the hydrated mucous gel covering the corneal surface in its measurement.

Future studies should focus on the investigation of the effects of corneal parameters on assessment of ECD within specific age groups. Although the horizontal corneal diameter is a clear indicator for ECD during infancy and childhood up to early teenage years, the present study suggests that it is not correlated with ECD assessment in the elderly. However, unlike in the young cornea, corneal thickness plays a significant role when ECD is measured in the older eye. Although corneal thickness is generally seen as an independent dimension that does not change significantly after infancy, a small decrease in value throughout life has been previously suggested. The effects of such changes in corneal thickness on the assessment of declining ECD during life should be investigated within different age groups and populations in order to better understand differences in ECD and the implications of such differences between individuals.

ACKNOWLEDGEMENTS

The authors would like to thank Dr Andrew Riley for his contribution to the research, particularly for the collection of data and clinical assessment of patients.

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Accepted for publication 13 June 2003

REFERENCES


Table 2

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Methodology</th>
<th>Age (years)</th>
<th>Mean (SD) corneal thickness (mm)</th>
<th>Mean (SD) ECD (cells/μm²)</th>
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<tr>
<td>Savo et al</td>
<td>1982</td>
<td>Ultrasound pachymetry</td>
<td>1.6–7</td>
<td>0.524 (central)</td>
<td>1053 (3067)**</td>
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<td>1997</td>
<td>Non-contact specular microscopy</td>
<td>70.7 (4.5)</td>
<td>0.520 (0.052) (central)</td>
<td>2551 (345)</td>
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</tr>
<tr>
<td>Müller, 2000</td>
<td></td>
<td>Optical low coherence reflectometer and ultrasound pachymetry</td>
<td>101.3 (1.3)</td>
<td>0.528 (0.033) (central)</td>
<td>3542 (510)</td>
<td>No</td>
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<tr>
<td>Ventura et al, 2001</td>
<td></td>
<td>Contact specular microscopy</td>
<td>71 (15)</td>
<td>0.537 (0.027) (central)</td>
<td>Approx. 2400</td>
<td>Yes, (p = 0.019, r=0.32)</td>
</tr>
</tbody>
</table>

*Corneal graft. †Pre-cataract. ‡Normal cornea. §Ultrasound pachymetry. *Optical low coherence reflectometer and ultrasound pachymetry. **Non-contact specular microscopy.
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doi: 10.1136/bjo.2003.019315

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