The keratoconic cornea is characterized by apical thinning or anterior protrusion that can result in broader corneal thinning. The progression of anterior protrusion is thought to reflect progression of the disease itself and has been proposed to be caused by slippage of collagen lamellae in the corneal stroma. We previously detected flattening and spreading of collagen lamellae in the keratoconic cornea by second-harmonic generation imaging microscopy. Such changes to collagen lamellae are thought to result in a loss of corneal rigidity that promotes corneal protrusion and corneal thinning. Studies of corneal fibroblasts derived from individuals with keratoconus have suggested that increased oxidative stress may trigger the activation of matrix metalloproteinases or inhibition of tissue inhibitors of matrix metalloproteinases and thereby promote collagen degradation by these cells that may be related to thinning of the corneal stroma. Stromal inhibition of tissue inhibitors of matrix metalloproteinases and changes to collagen lamellae are thought to result in a loss of corneal rigidity that promotes corneal protrusion and thinning. Studies of corneal fibroblasts derived from individuals with keratoconus have suggested that increased oxidative stress would be expected to result in a loss of stromal mass. Evaluation of CV might therefore provide important information with regard to the pathophysiology or natural progression of keratoconus.

CV can be calculated from a series of corneal sectional images that allow measurement of corneal thickness (CT) as well as anterior and posterior curvature. The volume of the keratoconic cornea has been determined with the use of several clinical examination tools including a Scheimpflug camera and anterior segment–optical coherence tomography (AS-OCT). Studies with a Scheimpflug camera have found that CV is decreased in eyes with keratoconus or subclinical keratoconus. Given that an increase in anterior corneal curvature is thought to reflect progression of keratoconus, we have now evaluated the relations among corneal curvature, CT, and CV in the keratoconic cornea as well as the relations between these clinical parameters and the severity of corneal scarring.

**Methods**

**Subjects**

This clinical study was approved by the Internal Review Board of Ohshima Eye Hospital (approval no. OEH2017-06) and adhered to the tenets of the Declaration of Helsinki.
consent was obtained from all subjects. Individuals diagnosed with keratoconus at Ohshima Eye Hospital from March 2014 through June 2018 were enrolled in the study. Patients attending the hospital for unrelated ocular conditions were recruited as control subjects. The diagnostic criteria for keratoconus included corneal irregular astigmatism and asymmetric anterior protrusion of the lower central region of the cornea as evaluated by corneal topography. Individuals with severe corneal scarring that impeded evaluation of the full thickness of the cornea, with suspected pellucid degeneration as evaluated by corneal topography, with severe stromal edema due to acute hydrops, or with other corneal conditions such as corneal trauma or corneal leucoma were excluded from enrollment as keratoconus subjects. Enrollment criteria for control subjects included the absence of astigmatism or the presence of regular astigmatism of <2.0 diopter, the absence of corneal lesions, and the absence of a history of corneal disease. None of the subjects in either group had undergone any ocular surgery such as refractive surgery, corneal cross-linking, insertion of intracorneal ring, or keratoplasty. All AS-OCT images and data were reviewed to confirm that individuals whose cornea had the appearance of a cone or who showed posterior irregular astigmatism were not enrolled as control subjects. A total of 288 eyes of 174 individuals with keratoconus (114 males and 60 females with a mean ± SD age of 42.8 ± 14.8 years and age range of 14–77 years) and 114 eyes of 57 control subjects (13 males and 44 females with a mean ± SD age of 42.4 ± 11.9 years and age range of 22–66 years) were enrolled in the study.

**Examination**

All subjects were examined by AS-OCT (CASIA; Tomey, Nagoya, Japan) at least three times by operators masked to the purpose of the examination. The masked examiners selected one of the three data sets with an adequate vertical iris angle relative to the cornea. The region corresponding to the cornea was automatically traced. The central corneal region with a diameter of 8 mm was automatically selected. (A) Schematic image for calculation of CV from this central region of the cornea. The 16 continuous corneal slices formed the three-dimensional corneal shape, and the volume of the central 8-mm-diameter region was calculated as described in Supplementary Information.

**Statistical Analysis**

Anterior refractive value, posterior refractive value, CT, and CV were compared among corneal stromal scarring patterns with a mixed-effects model. Statistical analysis was performed by a third party (Toshihito Furukawa, Biostatistical Research Co. Ltd., Tokyo, Japan). A P value of <0.05 was considered statistically significant.

**RESULTS**

The distributions of anterior refractive value, posterior refractive value, CT, and CV in the control and keratoconus (both with or without scarring) groups of eyes are shown in Figure 2. The mean ± SD and range of anterior refractive value in the control group and the keratoconus group were 48.5 ± 1.4 D and 42.5 to 52.5 D, and 56.7 ± 9.0 D and 42.5 to 100.0 D, respectively (Figs. 2A, 2E). The corresponding values for posterior refractive value were −6.2 ± 0.2 D and −5.83 to −6.83 D, and −7.9 ± 1.5 D and −5.45 to −11.72 D, respectively (Figs. 2B, 2F); those for CT were 524.1 ± 51.9 μm and 375 to 625 μm, and 408.6 ± 87.3 μm and 100 to 600 μm, respectively (Figs. 2C, 2G); and those for CV were 33.0 ± 1.9 mm3 and 26 to 37 mm3, and 31.7 ± 2.0 mm3 and 26 to 39 mm3, respectively (Figs. 2D, 2H). Overall, these findings indicated that anterior refractive value, posterior refractive value, and CT were narrowly distributed in the control group but widely distributed in the keratoconus group, whereas the distribution pattern of CV was similar for the two groups.

To investigate the relations among corneal curvature, CT, and CV, we constructed scatter plots (Fig. 3). We first plotted CT versus CV for all the study subjects (Fig. 3A). CT and CV were well correlated in the control group (regression curve of $y = 15.972x - 3.1058$, $R^2 = 0.8668$) but not in the keratoconus group ($y = -21.48x - 271.81$, $R^2 = 0.2422$). We then constructed such plots separately for keratoconic eyes classified according to their anterior refractive value (Figs. 3B–F). The regression...
FIGURE 2. Frequency distribution of (A, E) anterior and (B, F) posterior refractive values, (C, G) CT, and (D, H) CV for (A–D) control and (E–H) keratoconic eyes.
FIGURE 3. Scatter plots of CT versus CV according to anterior or posterior refractive value. (A) Plot for all control (open circles) and keratoconic (closed black circles) eyes. (B–F) Plots for all control eyes as well as for keratoconic eyes according to the indicated ranges of anterior refractive (Ant. refract.) value. (G–K) Plots for all control eyes as well as for keratoconic eyes according to posterior refractive (Post. refract.) value. Green and red closed circles indicate the median of CT and CV for control eyes and keratoconic eyes, respectively.
curves for CT versus CV for keratoconic eyes with anterior refractive values of \( \leq 50 \) D, between 50 and 54 D, between 54 and 60 D, between 60 and 68 D, or \( > 68 \) D were \( y = 15.78x – 20.921 \) \((R^2 = 0.702)\), \( y = 14.865x – 22.536 \) \((R^2 = 0.373)\), \( y = 17.786x – 169.54 \) \((R^2 = 0.3491)\), \( y = 20.927x – 321.88 \) \((R^2 = 0.3569)\), and \( y = 13.688x – 147.32 \) \((R^2 = 0.2461)\), respectively. Similar plots of CT versus CV according to posterior refractive value (Figs. 3G–K) yielded regression curves of \( y = 17.283x – 62.74 \) \((R^2 = 0.6188)\), \( y = 16.785x – 64.6 \) \((R^2 = 0.7498)\), \( y = 18.109x – 140.39 \) \((R^2 = 0.8213)\), \( y = 23x – 335.09 \) \((R^2 = 0.7029)\), and \( y = 17.577x – 248.25 \) \((R^2 = 0.26)\) for \( \geq 6.6 \) D, between \(-6.6 \) and \(-7.2 \) D, between \(-7.2 \) and \(-8.0 \) D, between \(-8.0 \) and \(-10.0 \) D, and \(< -10.0 \) D, respectively. For keratoconic eyes with anterior refractive values of \( \leq 50 \) D, between 50 and 54 D, between 54 and 60 D, between 60 and 68 D, or \( > 68 \) D, the median values for CV and CT were 32.0 mm\(^3\) and 487 \( \mu \)m, 31.7 mm\(^3\) and 452 \( \mu \)m, 31.9 mm\(^3\) and 409 \( \mu \)m, 31.6 mm\(^3\) and 330 \( \mu \)m, and 30.8 mm\(^3\) and 287 \( \mu \)m, respectively. For keratoconic eyes with posterior refractive values of \( \geq 6.6 \) D, between \(-6.6 \) and \(-7.2 \) D, between \(-7.2 \) and \(-8.0 \) D, between \(-8.0 \) and \(-10.0 \) D, and \(< -10.0 \) D, the median values of CV and CT were 31.8 mm\(^3\) and 487 \( \mu \)m, 31.6 mm\(^3\) and 456 \( \mu \)m, 31.9 mm\(^3\) and 432 \( \mu \)m, 32.1 mm\(^3\) and 374 \( \mu \)m, and 30.3 mm\(^3\) and 268 \( \mu \)m, respectively. The median CV and CT values thus decreased as the anterior refractive value or posterior refractive value increased or decreased, respectively, indicating that CV and CT declined in a correlated manner with the decrease in CT occurring before that in CV according to the progression of keratoconus.

To investigate the relation of corneal scarring to corneal shape, thickness, or volume, we classified AS-OCT images of keratoconic eyes on the basis of the pattern of stromal scarring. Representative AS-OCT images for the different scarring patterns are shown in Figure 4. All AS-OCT images of the control subjects were classified as Normal. Totals of 114, 168, 44, and 76 eyes were classified into the Normal, Scar(–), Ant. Scar, and Ant./Post. Scar subgroups, respectively. The distributions of anterior refractive value, posterior refractive value, CT, and CV for each subgroup are shown in Figure 5. Anterior refractive value increased with progression from Normal to Scar(–) to Ant. Scar to Ant./Post. Scar. The distribution of anterior refractive value differed significantly between each pair of scarring patterns (Table 1). Posterior refractive value decreased with progression from Normal to Scar(–) to Ant. Scar to Ant./Post. Scar. The distribution of posterior refractive value also differed significantly between each pair of scarring patterns (Table 2). Similarly, both CT (Fig. 5C) and CV (Fig. 5D) decreased with progression from Normal to Scar(–) to Ant. Scar to Ant./Post. Scar. The distribution of CT differed significantly between each pair of scarring subgroups (Table 3), whereas that of CV differed significantly between each pair of scarring subgroups with the exception between Scar(–) and Ant. Scar. Ant. Scar (Table 4), suggesting that anterior scarring may not affect CV.

We also constructed scatter plots of CT versus CV according to scarring pattern (Fig. 6). The distribution and median values of these parameters for the Scar(–) group were similar to those for the Normal group (Fig. 6A). The distribution of the Ant. Scar subjects shifted toward lower CT values (Fig. 6B), whereas that of the Ant./Post. Scar subjects shifted toward both lower CT and CV values (Fig. 6C), again indicating that both CT and CV decline with progression of scar formation, with the decline in CT preceding that in CV.

| Table 1. Statistical Analysis for Comparison of Anterior Refractive Value Among Corneal Scarring Pattern Groups |
|--------------------------------------------------|----------------------------------|--|------------------|------------------|------------------|------------------|
| Scarring Pattern | Mean 95% CI | Estimated Value* 95% CI | P Value | Estimated Value* 95% CI | P Value | Estimated Value* 95% CI | P Value |
| Normal | 48.55 47.48–49.62 | Normal | 51.7 50.86–52.21 | 3.15 1.79–4.51 | <0.0001 | 6.53 4.72–8.34 | <0.0001 | 8.43 6.39–10.47 | <0.0001 |
| KC | Scar(–) | Ant. Scar | Ant./post. scar | 58.23 56.61–59.85 | 9.68 7.74–11.62 | <0.0001 | 14.96 13.47–16.45 | <0.0001 | 8.43 6.39–10.47 | <0.0001 |
| Normal | 48.55 47.48–49.62 | Estimated Value* 95% CI | P Value | Estimated Value* 95% CI | P Value | Estimated Value* 95% CI | P Value |
| Normal | 51.7 50.86–52.21 | 3.15 1.79–4.51 | <0.0001 | 6.53 4.72–8.34 | <0.0001 | 8.43 6.39–10.47 | <0.0001 |
| Ant. Scar | 58.23 56.61–59.85 | 9.68 7.74–11.62 | <0.0001 | 14.96 13.47–16.45 | <0.0001 | 8.43 6.39–10.47 | <0.0001 |
| Ant./post. scar | 66.66 65.41–67.91 | 18.11 16.47–19.75 | <0.0001 | 14.96 13.47–16.45 | <0.0001 | 8.43 6.39–10.47 | <0.0001 |

* Estimated value of the average difference between groups.

CI, confidence interval; KC, keratoconus.
We have here analyzed the relations among anterior or posterior refractive value, CT, and CV in normal and keratoconic corneas. We found that the progression of scar formation in the keratoconic cornea was associated with the progression of corneal deformation, corneal thinning, and loss of CV. Furthermore, loss of CT or CV was associated with disease progression, with the decrease in CV being detected at the advanced stage of the disease.

We found that the regression curves for CT versus CV were linear for control eyes and eyes with mild keratoconus, whereas the curves changed to reflect a decreased thickness in moderately keratoconic eyes and then a decreased thickness and volume in severely keratoconic eyes, indicated by a shift in median values of each distribution (Fig. 3). These findings suggested that corneal thinning in mild and moderate keratoconus occurred without a decrease in CV, whereas the change in shape would not result in a loss of CV. Our observations suggest that such external expansion is characteristic of mild to moderate keratoconus, and that a decrease in CV is indicative of disease progression to an advanced stage, as previously suggested.

The distribution of anterior corneal curvature, posterior curvature, and CT for keratoconic eyes was wider than that for control eyes, whereas the distribution of CV was similar for both types of eye. These findings suggest that CV is maintained in most keratoconic corneas even though other corneal parameters may change markedly, again consistent with the notion that CV declines only in eyes with severe keratoconus.

Examination of the relation between corneal stromal scarring pattern in keratoconus and anterior or posterior refractive value, CT, or CV revealed that the extent of scarring was associated with that of corneal curvature, corneal thinning, and the loss of CV. Disruption of Bowman’s layer has previously been associated with corneal scarring in keratoconus. A review of all AS-OCT images in the present study failed to reveal a scarring pattern characterized by

**Figure 5.** Box-and-whisker plots showing the distribution of anterior and posterior refractive values, CT, and CV in the Normal (A), Scar(-) (B), Ant. Scar (C), and Ant./Post. Scar (D) subgroups of eyes. Boxes indicate the quartile range, with the top of each box representing the third quartile, the bottom the first quartile, and the middle line the median value; whiskers indicate maximum and minimum value; open circles indicate outliers; and cross marks indicate the mean value.
### Table 2. Statistical Analysis for Comparison of Posterior Refractive Value Among Corneal Scarring Pattern Groups

<table>
<thead>
<tr>
<th>Scarring Pattern</th>
<th>Mean</th>
<th>95% CI</th>
<th>Estimated Value*</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>6.21</td>
<td>-6.4 to -6.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KC</td>
<td>6.99</td>
<td>-7.13 to -6.84</td>
<td>-0.78</td>
<td>-1.02 to -0.54</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ant. scar</td>
<td>8.52</td>
<td>-8.79 to -8.24</td>
<td>-2.3</td>
<td>-2.64 to -1.97</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ant./post. scar</td>
<td>9.57</td>
<td>-9.78 to -9.36</td>
<td>-3.36</td>
<td>-3.64 to -3.07</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

CI, confidence interval; KC, keratoconus.
* Estimated value of the average difference between groups.

### Table 3. Statistical Analysis for Comparison of CT Among Corneal Scarring Pattern Groups

<table>
<thead>
<tr>
<th>Scarring Pattern</th>
<th>CT (µm)</th>
<th>Mean</th>
<th>95% CI</th>
<th>Estimated Value*</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>522.95</td>
<td>510.3 to 535.61</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KC</td>
<td>456.72</td>
<td>447.57 to 465.87</td>
<td>-66.23</td>
<td>-81.85 to -50.62</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Ant. scar</td>
<td>393.67</td>
<td>377.56 to 409.78</td>
<td>-129.28</td>
<td>-149.77 to -108.80</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Ant./post. scar</td>
<td>312.70</td>
<td>299.83 to 325.57</td>
<td>-210.25</td>
<td>-228.30 to -192.20</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

CI, confidence interval; KC, keratoconus.
* Estimated value of the average difference between groups.
posterior scarring without anterior scarring. We also found that anterior stromal scarring was associated with moderate changes in corneal parameters, whereas the presence of both anterior and posterior scarring was associated with more pronounced changes in these parameters. A multicenter study of the natural history of keratoconus found a correlation between corneal scarring and corneal curvature, consistent with our current results. Our findings thus suggest that anterior stromal scarring occurs before posterior stromal scarring. Anterior scarring is likely induced by external influences such as the wearing of hard contact lenses and rubbing of the cornea, whereas posterior stromal scarring is induced by disruption of Descemet's membrane. It has remained unclear whether stromal scarring causes stromal shrinkage and a consequent change in corneal curvature or whether stromal scarring is a cause or a result of the progression of keratoconus. However, stromal scarring is recognized as an important indicator of the progression of keratoconus.

**Figure 6.** Scatter plots of CT versus CV according to scarring pattern. Plots are shown for all control eyes (Normal pattern, open circles) and for keratoconic eyes (closed black circles) showing the Scar(−) (A), Ant. Scar (B), or Ant./Post. Scar (C) pattern. Green and red closed circles indicate the median of CT and CV for control eyes and keratoconic eyes, respectively.
Our results now provide insight into the natural course of keratoconus progression (Fig. 7). In the mild stage of keratoconus, corneal curvature is increased without corneal thinning. At the moderate stage, corneal curvature is further increased in association with corneal thinning and anterior stromal scarring. Finally, at the severe stage, corneal curvature is increased even further in association with more widespread corneal thinning, as suggested by a loss of CV, and with anterior and posterior scar formation. Our findings thus suggest that substantial stromal degradation does not occur at the early stages of keratoconus but does so at the advanced stage.

Keratoconus is a common corneal disease and has been much studied, but its pathogenesis has not been fully revealed. Our clinical study now suggests one possible mechanism for the progression of keratoconus that may provide a basis for further studies and the potential development of new treatments.

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References