Lamina Cribrosa and Peripapillary Sclera Histomorphometry in Normal and Advanced Glaucomatous Chinese Eyes with Various Axial Length

Ruojin Ren,1,2 Ningli Wang,1,2 Bin Li,*,1 Liaoqing Li,1 Fei Gao,1 Xiaolin Xu,1 and Jost B. Jonas*1,3

PURPOSE. To measure laminar and peripapillary scleral architecture in normal and glaucomatous Chinese eyes with normal and those with elongated axial length.

METHODS. The histomorphometric investigation included a normal group (nonaxially elongated eyes) of 40 human globes (40 patients; mean age, 41.3 ± 13.4 years; range, 15–68) enucleated because of malignant choroidal melanoma, a glaucomatous group (non-axially elongated eyes) of 55 eyes (55 patients; age, 43.5 ± 20.3 years; range, 12–88) enucleated because of painful secondary angle-closure glaucoma, and a group of 26 glaucomatous globes (glaucomatous elongated axial length group; 26 patients; age, 29.0 ± 14.4 years; range, 12–60) with an axial length > 27.5 mm. Anterior-posterior histologic sections were morphometrically evaluated.

RESULTS. The lamina cribrosa was significantly ($P < 0.001$) thicker in the normal group than in the glaucomatous group, in which it was significantly ($P < 0.001$) thicker than in the glaucomatous elongated-length group. Lamina cribrosa thickness decreased significantly with increasing axial length ($P < 0.001$) and presence of glaucoma ($P < 0.001$). Peripapillary scleral thickness close to the optic nerve scleral canal and just outside of the optic nerve meninges decreased significantly with increasing axial length ($P = 0.04$ and $P = 0.02$, respectively). Peripapillary scleral thickness did not vary significantly between the glaucomatous group and the normal group. The distance between the intraocular space and cerebrospinal fluid space was ($P < 0.001$) shorter in the two glaucomatous groups than in the normal group.

CONCLUSIONS. Lamina cribrosa thickness and peripapillary sclera thickness decreased significantly with axial length, in addition to a glaucoma-related thinning of the lamina cribrosa. In non-axially elongated eyes, the peripapillary sclera thickness did not vary significantly between glaucomatous eyes and normal eyes. (Invest Ophthalmol Vis Sci. 2009;50:2175–2184) DOI: 10.1167/iovs.07-1429

Recent studies of the morphology of the optic nerve head in normal and glaucomatous monkey and human eyes have markedly widened the knowledge of the biomechanics and anatomy of the optic nerve head.1–11 These investigations have contributed to a new understanding of the mechanical interplay between the lamina cribrosa and the sclera and how these structures potentially influence the susceptibility of the optic nerve head axons to glaucomatous damage.5–9

Both the histomorphometric and modeling studies to date emphasize the importance of the architecture (shape and thickness) and material properties (stiff, brittle, and compliant) of the peripapillary sclera.1–4 These studies suggest that the peripapillary sclera provides the boundary conditions for the lamina cribrosa, which acts like a trampoline within the scleral portion of the neural canal. After an acute elevation of intraocular pressure, the structural stiffness of the peripapillary sclera (the combination of peripapillary scleral thickness and material properties) directly influences how the lamina deforms. A compliant sclera (thin and/or elastic) allows the scleral canal to expand, which tightens the laminar beams within the canal and thereby increases their resistance to posterior deformation. In contrast, a rigid peripapillary sclera (thick and/or stiff) allows less expansion of the canal or none at all, forcing the structural stiffness of the lamina alone to bear the intraocular pressure–related stress. Taken together, these concepts emphasize the importance of characterizing both components of peripapillary scleral structural stiffness (geometric and material properties) in attempting to understand the effect of intraocular pressure on the optic nerve head.

Given the importance of the lamina cribrosa and peripapillary sclera in optic nerve head biomechanics1–4 and the lack of peripapillary scleral thickness measurements in previous studies in Caucasians,9,11 the purpose of the present investigation was to measure laminar and peripapillary scleral architecture in normal eyes, glaucomatous eyes with a normal axial length, and glaucomatous eyes with an elongated axial length.

METHODS

The study protocol complied with the guidelines of the Declaration of Helsinki for the study of human tissue. This retrospective histomorphometric study consisted of three study groups: The normal group included 40 eyes (40 patients) that had been enucleated because of a malignant choroidal melanoma; the glaucomatous group consisted of 55 eyes (55 patients) that had been enucleated due to painful absolute secondary angle-closure glaucoma; and the glaucomatous elongated axial length group included 26 globes (26 patients), with an axial length longer than 27.5 mm, also enucleated due to painful secondary angle-closure glaucoma (Table 1). For determination of the axial length, a linear shrinkage factor of 15% was taken into account.12,13 In the normal group, intraocular pressure was within the normal range without antiglaucomatous medication. The malignant choroidal melanomas did not infiltrate the trabecular meshwork, directly nor indirectly, with migrating cells. The parapapillary region was free of tumor. Visual acuity depended on the degree of cataract, vitreous...
Approximate age at known onset of IOP increase (y) — 1.8
Central corneal thickness (mm) 0.51 — 0.07
Vertical diameter (mm) 10.7 ± 0.7
Horizontal diameter (mm) 11.2 ± 0.7
Axial length (mm) 23.9 ± 1.3
Corneal diameter (mm) 10.7 ± 0.7

In the glaucomatous and the glaucomatous elongated axial length groups, vision was completely or almost completely lost. Enucleation became necessary, usually because of intractable pain that could not be treated by medication. The principal diagnosis in both glaucomatous groups was secondary angle-closure glaucoma due to either a penetrating corneal wound or diabetic neovascularization. In the case of the glaucomatous elongated axial length group, no data were available to determine whether the enucleated eye was axially elongated (and was therefore myopic) before the onset of intraocular pressure elevation or became axially elongated as a result of intraocular pressure elevation.

Immediately after enucleation, the globes were fixed in a solution of 4% formaldehyde. They remained in the fixation agent for approximately 24 to 48 hours, before they were further processed for histologic sectioning. In a routine histopathologic work-up, the axial length of the globes was measured after the fixation and before sectioning of the eyes. A ruler was used to determine the axial length measurements. The measurements were recorded in 1-mm steps. The globes were prepared in a routine manner for light microscopy. Since the reasons for enucleation differed between the glaucomatous eyes and the non-glaucomatous eyes, the eyes were opened in different directions. The reason for the enucleation of the glaucomatous eyes, independent of the axial length, was intolerable pain, so that the eyes of both glaucomatous groups were routinely opened in a vertical direction. The reason for the enucleation of the nonglaucomatous eyes was intraocular tumors. The location of the tumor was decisive for the direction of globe sectioning, so that the eyes of the normal group were opened in various directions. The further steps of the preparation of the globes did not vary between the study groups. An anterior–posterior segment going through the pupil and the optic nerve was cut out of the fixed globes. These segments were dehydrated in alcohol, imbedded in paraffin, sectioned for light microscopy, and stained by the hematoxylin-eosin method.

For further evaluation, one representative section running through the central part of the optic disc was selected out of a group of up to five slides. Since it was a retrospective study, and since for most eyes, the globes were no longer available to obtain additional slides, most of the histologic sections did not contain the central retinal vessel trunk. This limitation of the study held true in the same manner for all study groups. The histologic slides were digitized and morphometrically analyzed with a planimetric program (Motic Med 6.0, Xiamen Motic Software Engineering Co. Ltd, Xiamen, China). The scale of the tissue was determined by a calibrated control slide with a millimeter scale engraved. The resolution of the digitization of the histologic slides was 800 × 600 pixels.

We measured the following:

- The length of the inner and the outer surfaces of the lamina cribrosa (Figs. 1, 2);
- the thickness of the lamina cribrosa at five locations: in the center of the optic disc, at the two most peripheral parts of the lamina cribrosa, and in the two intermediary positions between the center and the most peripheral part of the lamina cribrosa (Figs. 1, 2);
- the thickness of the sclera at its thinnest part close to the optic nerve scleral canal and just outside of the optic nerve meninges (Fig. 3);
- the part of the sclera that merges with the optic nerve meninges, expressed as a percentage of the whole sclera thickness, roughly equal to the difference between the scleral thickness just outside of the optic nerve meninges minus the scleral thickness close to the optic nerve scleral canal, divided by the scleral thickness outside of the optic nerve meninges (Fig. 3); and
- the shortest distance between the intraocular space (inner surface line of the lamina cribrosa) and the cerebrospinal fluid space (Fig. 1).

The lamina cribrosa was defined as the collagenous tissue reaching from one end of the anterior opening of the optic nerve sclerad to the other end and showing a layered structure. On its anterior (vitread) side, it was easier to delineate than on its posterior side where it merged with the anterior portion of the optic nerve. The cerebrospinal fluid space was defined as the optically empty space behind the lamina cribrosa, located between the optic nerve itself and the optic nerve meninges. The sclera was the collagenous tissue between the outer opacities, and foveal involvement by the tumor. At the time when the eyes were enucleated, no other treatment modalities such as en-doresection of the tumor or radiologic brachytherapy were available, or the eyes were thought not to be suitable for tumor removal with respect to the location and size of the tumor.

In the glaucomatous and the glaucomatous elongated axial length groups, vision was completely or almost completely lost. Enucleation became necessary, usually because of intractable pain that could not be treated by medication. The principal diagnosis in both glaucomatous groups was secondary angle-closure glaucoma due to either a penetrating corneal wound or diabetic neovascularization. In the case of the glaucomatous elongated axial length group, no data were available to determine whether the enucleated eye was axially elongated (and was therefore myopic) before the onset of intraocular pressure elevation or became axially elongated as a result of intraocular pressure elevation.

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- the thickness of the sclera at its thinnest part close to the optic nerve scleral canal and just outside of the optic nerve meninges (Fig. 3);
- the part of the sclera that merges with the optic nerve meninges, expressed as a percentage of the whole sclera thickness, roughly equal to the difference between the scleral thickness just outside of the optic nerve meninges minus the scleral thickness close to the optic nerve scleral canal, divided by the scleral thickness outside of the optic nerve meninges (Fig. 3); and
- the shortest distance between the intraocular space (inner surface line of the lamina cribrosa) and the cerebrospinal fluid space (Fig. 1).
surface of the globe and the beginning of the choroid. For all measurements, the minimum distances were generally those recorded. In addition, we measured the horizontal and vertical diameter of the cornea and the central corneal thickness. The information on the known duration of the elevation of intraocular pressure was taken from the charts.

Statistical analysis was performed with a commercially available statistical software package (SPSS for Windows, ver. 16.0; SPSS, Chicago, IL). The data were presented as the mean and standard deviations as well as medians and ranges. For the comparison of the study groups, statistical tests (Mann-Whitney test or Student’s t-test) for unpaired samples were applied. For the comparison of parameters within the same optic nerve head, statistical tests (Wilcoxon test or Student’s t-test) for paired samples were applied. If there were two or more variables associated with the dependent parameter, a multivariate analysis was performed. The level of significance was 0.05 in all statistical tests.

To evaluate the reproducibility of the technique, 10 randomly selected histologic optic disc sections were re-evaluated five times. The reproducibility was determined as the coefficient of variation defined as the ratio of the mean of the standard deviations of the re-evaluations divided by the mean of the means. The same person (RR) performed all measurements including the reproducibility study.

**RESULTS**

**Patient Demographics**

The mean age of the glaucomatous elongated axial length group (27.9 ± 11.2 years) was significantly lower than the age of the normal group (41.3 ± 13.4 years; \( P < 0.001 \)) and the age of the glaucoma group (43.3 ± 20.3 years; \( P < 0.001 \)), whereas the two latter groups did not vary significantly (\( P = 0.57 \)) in age (Table 1). No data on the relative magnitude of the intraocular pressure insult before enucleation were available for the glaucomatous groups.

**Anterior and Posterior Laminar Surface Lengths**

The length of the inner surface line of the lamina cribrosa was significantly longer in the glaucomatous elongated axial length group than in the glaucomatous group (\( P < 0.001 \)), in which it was significantly (\( P < 0.001 \)) longer than in the normal group (Table 2). In a similar manner, the length of the outer surface line of the lamina cribrosa was significantly (\( P = 0.02 \)) longer in the glaucomatous elongated axial length group than in the glaucomatous group, in which it was significantly (\( P = 0.02 \)) longer than in the normal group (Table 2). The outer lamina cribrosa length was significantly (\( P < 0.001 \)) longer than the inner lamina cribrosa length in all three study groups.

Because not all the histologic slides went exactly through the center of the optic nerve head, two additional parameters were calculated to correct for this inaccuracy. The parameters were determined as the inner lamina cribrosa surface line length divided by the length of Bruch’s membrane opening and as the outer lamina cribrosa surface line length divided by the length of Bruch’s membrane opening. The length of Bruch’s membrane opening was defined as the distance between both ends of Bruch’s membrane at the margin of the optic nerve head. Both parameters of the lamina cribrosa surface length were slightly larger in the glaucomatous group than in the glaucomatous elongated axial length group (\( P = 0.075 \) and \( P = \))
Table 2. Histomorphometric Measurements of the Lamina Cribrosa Surface Length

<table>
<thead>
<tr>
<th></th>
<th>Normal Group</th>
<th>Glaucoma Group</th>
<th>Glaucomatous Elongated Axial Length Group</th>
<th>( P^* )</th>
<th>( P^† )</th>
<th>( P^‡ )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n ) (eyes/patients)</td>
<td>40</td>
<td>55</td>
<td>26</td>
<td>&lt;0.001</td>
<td>0.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Inner LSL (( \mu m ))</td>
<td>1613 ± 396</td>
<td>2164 ± 677</td>
<td>2496 ± 692</td>
<td>&lt;0.001</td>
<td>0.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median (range)</td>
<td>1665 (853–2593)</td>
<td>2041 (913–3950)</td>
<td>2459 (912–3786)</td>
<td>&lt;0.001</td>
<td>0.02</td>
<td>0.001</td>
</tr>
<tr>
<td>Outer LSL (( \mu m ))</td>
<td>1977 ± 399</td>
<td>2341 ± 661</td>
<td>2674 ± 683</td>
<td>&lt;0.001</td>
<td>0.02</td>
<td>0.001</td>
</tr>
<tr>
<td>Median (range)</td>
<td>2001 (1025–2747)</td>
<td>2178 (1287–4028)</td>
<td>2695 (1122–3945)</td>
<td>&lt;0.001</td>
<td>0.02</td>
<td>0.001</td>
</tr>
<tr>
<td>BMO (( \mu m ))</td>
<td>1586 ± 266</td>
<td>1608 ± 262</td>
<td>2079 ± 364</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.65 (NS)</td>
</tr>
<tr>
<td>Inner LSL/BMO</td>
<td>1.01 ± 0.15</td>
<td>1.36 ± 0.32</td>
<td>1.20 ± 0.21</td>
<td>&lt;0.001</td>
<td>0.075 (NS)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Outer LSL/BMO</td>
<td>1.24 ± 0.14</td>
<td>1.45 ± 0.52</td>
<td>1.29 ± 0.20</td>
<td>0.29</td>
<td>0.04</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are the mean ± SD. LSL, lamina cribrosa surface length; BMO, Bruch’s membrane opening.
* Significance of differences between the glaucomatous elongated axial length group and the normal group.
† Significance of differences between the glaucomatous elongated axial length group and the glaucoma group.
‡ Significance of differences between the glaucoma and the normal group.

0.04, respectively), in which they were significantly (\( P < 0.001 \) and \( P = 0.29 \), respectively) higher than in the normal group (Table 2). The parameter for the inner lamina cribrosa surface length was significantly (\( P < 0.001 \)) shorter than the parameter for the outer lamina cribrosa surface length. The length of the opening of Bruch’s membrane as surrogate for the optic disc diameter was significantly (\( P < 0.001 \)) longer in the glaucomatous elongated axial length group than in the other two study groups which did not vary significantly in that parameter (Table 2).

**Lamina Cribrosa Thickness**

The lamina cribrosa was thickest in the normal group and progressively thinned in the glaucomatous group and glaucomatous elongated axial length group, with all three being significantly different from each other at all locations measured (Fig. 4).

Performing a multivariate analysis, with the lamina cribrosa thickness as the dependent parameter and axial length and the presence of glaucoma as independent parameters revealed that the lamina cribrosa thickness was significantly associated with a short axial length (\( P < 0.001 \); Fig. 5) and the absence of glaucoma (\( P < 0.001 \)) as an additional and independent factor. Within the normal group, the lamina cribrosa thickness was not significantly associated with the axial length (\( P = 0.54 \)).

In the univariate analysis, the lamina cribrosa was not significantly associated with age, in the whole study population (\( P = 0.43 \)) or considered separately in the normal group (\( P = 0.88 \)) and the glaucomatous elongated axial length group (\( P = 0.43 \)). In the glaucomatous group (non-axially elongated eyes), it was significantly associated with younger age (\( P = 0.02 \)). If a multivariate analysis was performed with central lamina cribrosa thickness as the dependent parameter and age, axial length, and study group or presence of glaucoma as independent parameters, the association between lamina cribrosa thickness and age was no longer statistically significant (\( P = 0.44 \)).

**Peripapillary Scleral Thickness**

The thickness of the sclera just outside of the optic nerve meninges (\( P = 0.99 \)) and the thickness of the thinnest part of the peripapillary sclera close to the optic nerve scleral canal (\( P = 0.41 \)) did not vary significantly between the normal group and the glaucomatous group. Correspondingly, the part of the sclera passing into the optic nerve meninges, expressed as a percentage of the whole sclera thickness, did not vary significantly between the normal group and the glaucomatous group (49\% ± 12\% vs. 46\% ± 11\%; \( P = 0.25 \)). In a similar manner, the part of the sclera passing into the optic nerve meninges, expressed as a percentage of the whole sclera thickness, did not vary between the glaucomatous elongated axial length group (49\% ± 13\%) and the latter groups (\( P = 0.86 \) and \( P = 0.47 \), respectively).

The sclera just outside of the optic nerve meninges was significantly thinner in the glaucomatous elongated axial length group than in the other two study groups (\( P = 0.03 \) and \( P = 0.04 \), respectively). In a similar manner, the sclera inside of the optic nerve meninges close to the optic nerve scleral canal was slightly, but not significantly (\( P = 0.09 \)), thinner in the glaucomatous elongated axial length group than in the normal group.

Performing a multivariate analysis, with the peripapillary scleral thickness as the dependent parameter and axial length and the presence of glaucoma as independent parameters revealed that peripapillary scleral thickness was not significantly associated with the presence of glaucoma (\( P = 0.34 \) for scleral thickness at the disc border; and \( P = 0.89 \) for scleral thickness just outside of the meninges). The part of the sclera passing into the optic nerve meninges, expressed as a percentage of the whole sclera thickness, did not vary significantly between all three study groups.

The thickness of the posterior sclera just outside of the optic nerve meninges was significantly and inversely associated with axial length (\( P = 0.02 \); 95\% confidence interval [CI] of the slope of the regression line: \(-23.2 \) to \(-1.8 \)). In a similar manner, there was a tendency toward a lower thickness of the peripapillary sclera close to the optic nerve scleral canal in eyes with longer axial axis. This association, however, was not statistically significant (\( P = 0.23 \)). Performing a multivariate analysis with the scleral thickness close to the optic nerve scleral canal as the dependent variable and axial length and age as independent variables and including only the glaucomatous elongated axial length group and the normal group revealed that a thinner scleral thickness close to the optic nerve scleral canal was significantly associated with axial length (\( P = 0.04 \)).

**Shortest Translaminar Distance**

The shortest distance between the intraocular space (i.e., the inner surface of the lamina cribrosa) and the cerebrospinal fluid pressure space (i.e., outer surface of the pia mater) was significantly (\( P < 0.001 \)) longer in the normal group than in the glaucomatous group and or the glaucomatous elongated axial length group (Fig. 6). In all eyes included in the study, the region with the shortest distance between the intraocular pres-
sure space and the cerebrospinal fluid pressure space was located in the periphery of the optic nerve head.

**Reproducibility**

The coefficient of variation for the reproducibility of the measurements of the central lamina cribrosa thickness was 5.1%, for the thickness of sclera at the optic disc border 14.8%, for the scleral thickness just outside of the optic nerve meninges 7.7%, for the distance between the intraocular space and the cerebrospinal fluid space 15.2%, and for the length of the anterior and posterior surface line of the lamina cribrosa 23.2% and 19.1% (Table 3).

**FIGURE 4.** Box plots showing the lamina cribrosa thickness measurements and the shortest translaminar distance measurements in eyes of the normal group, glaucomatous group (non-axially elongated eyes), and glaucomatous elongated axial length group. The lamina cribrosa was significantly ($P < 0.001$) thicker in the normal group than in the glaucomatous group, in which it was significantly ($P < 0.001$) thicker than in the glaucomatous elongated axial length group. The shortest translaminar distance was significantly ($P < 0.001$) longer in the normal group than in the glaucomatous group and than in the glaucomatous elongated axial length group. The two latter groups did not vary significantly ($P = 0.48$).

**FIGURE 5.** Scattergram showing the correlation between central lamina cribrosa thickness and axial length; correlation coefficient $r = -0.49$; $P < 0.001$; the graph shows the normal group in which the lamina cribrosa was thicker than in the glaucoma group, in which, in turn, the lamina cribrosa was thicker than in the glaucomatous elongated axial length group.
Other Measurements

The glaucomatous elongated axial length group did not vary significantly from the glaucomatous group and the normal group in horizontal corneal diameter ($P = 0.12$ and $P = 0.08$, respectively), in vertical corneal diameter ($P = 0.27$ and $P = 0.07$), or in central corneal thickness ($P = 0.55$ and $P = 0.63$; Table 1). In a parallel manner, the glaucomatous elongated axial length group and the glaucomatous group did not differ significantly in the known duration of elevation of intraocular pressure ($P = 0.83$; Table 1).

The mean age of the patients in the two glaucoma groups when the known elevation of intraocular pressure started was significantly ($P < 0.001$) higher in the glaucomatous group than in the glaucomatous elongated axial length group (41.4 ± 20.4 years versus 26.0 ± 11.4 years; Table 1). The known duration of the increase in intraocular pressure did not vary significantly between the two groups with glaucoma ($P = 0.75$).

The present study is the first to characterize laminar and peripapillary scleral architecture in normal eyes, glaucomatous eyes with a normal axial length, and glaucomatous Chinese eyes with a markedly elongated axial length. Its principal findings are as follows. First, laminar thickness: the lamina cribrosa was significantly ($P < 0.001$) thicker in the normal group than in the glaucomatous group, in which it was significantly ($P < 0.001$) thicker than in the glaucomatous elongated axial length group. The lamina cribrosa thickness decreased significantly ($P < 0.001$) with increasing axial length and presence of glaucoma ($P < 0.001$). Correspondingly in a multivariate analysis, a thin lamina markedly elongated axial length, the lamina cribrosa was significantly thinner than in eyes with a normal axial length, thus decreasing the distance between the intraocular space and the cerebrospinal fluid space.11 In the peripapillary region, a loss of retinal pigment epithelium cells and photoreceptors was described.14

DISCUSSION

Previous histomorphometric studies on Caucasian globes have revealed that in eyes with end-stage glaucoma compared with normal eyes, the lamina cribrosa was significantly thinner, that the part of the outer lamina cribrosa surface directly exposed to the pia mater and indirectly exposed to the cerebrospinal fluid space was significantly wider and that the shortest distance between the intraocular space and the cerebrospinal fluid space was significantly shorter.9 These studies also showed that in eyes with a markedly elongated axial length, the lamina cribrosa was significantly thinner than in eyes with a normal axial length, thus decreasing the distance between the intraocular space and the cerebrospinal fluid space.11 In the peripapillary region, a loss of retinal pigment epithelium cells and photoreceptors was described.14

Table 3. Reproducibility of the Histomorphometric Measurements of the Optic Nerve Head

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measurements of Lamina cribrosa thickness</td>
<td>5.1</td>
</tr>
<tr>
<td>Scleral thickness at the optic disc border</td>
<td>14.8</td>
</tr>
<tr>
<td>Scleral thickness just outside of the optic nerve meninges</td>
<td>7.7</td>
</tr>
<tr>
<td>Distance between intraocular space and cerebrospinal fluid space</td>
<td>15.2</td>
</tr>
<tr>
<td>Length of the anterior surface line of the lamina cribrosa</td>
<td>23.2</td>
</tr>
<tr>
<td>Length of the posterior surface line of the lamina cribrosa</td>
<td>19.1</td>
</tr>
</tbody>
</table>

FIGURE 6. Box plots showing the inner and outer peripapillary sclera thickness measurements in eyes of the normal, glaucomatous (non-axially elongated eyes), and glaucomatous elongated axial length groups. The outer peripapillary sclera (just outside of the optic nerve meninges) was significantly thinner in the glaucomatous elongated axial length group than in the two other study groups ($P = 0.05$ and $P = 0.04$, respectively). In a similar manner, the sclera inside of the optic nerve meninges close to the optic nerve scleral canal was slightly, but not significantly ($P = 0.09$), thinner in the glaucomatous elongated axial length group than in the normal group.
cribrosa thickness was significantly associated with a long axial length \( (P < 0.001) \) and with the presence of glaucoma \( (P < 0.001) \) as an additional and independent factor. In agreement with the reduced thickness of the lamina cribrosa, the distance between the intraocular space and the cerebrospinal fluid pressure space was significantly \( (P < 0.001) \) shorter in the two glaucomatous groups than in the normal group. Second, parallel to the thinning of the lamina cribrosa in the glaucomatous elongated axial length group, the peripapillary scleral thickness decreased significantly with increasing axial length, independent of the presence or absence of glaucoma. Correspondingly, the peripapillary scleral thickness did not vary significantly between the glaucomatous group and the normal group. The part of the sclera passing into the optic nerve meninges (approximately 50%) did not vary significantly in its percentage of the whole sclera thickness between all three study groups. Third, the shortest distance between the intraocular space and the cerebrospinal fluid pressure space was significantly \( (P < 0.001) \) longer in the normal group than in the glaucomatous group and than in the glaucomatous elongated axial length group. In all eyes included in the study, the region with the shortest distance between the intraocular pressure space and the cerebrospinal fluid pressure space was located in the periphery of the optic nerve head.

The finding of a thinner lamina cribrosa in the glaucomatous eyes versus the normal eyes in Chinese confirms the finding of a similar study of Caucasians.\(^4\) In that study, one concluded that the thin lamina cribrosa in the eyes with end-stage glaucoma may be one of the reasons why in clinical studies the glaucoma susceptibility increased with advanced stage of the disease.\(^15,18\) The pathophysiologic basis for the hypothesis that a thinning of the lamina cribrosa may increase glaucoma susceptibility is that the lamina cribrosa forms the border between the intraocular space with a higher pressure and the retrobulbar space with the cerebrospinal fluid with a lower pressure.\(^19,20\) Since the lamina cribrosa is not indefinitely thin, the translaminar pressure difference leads to a translaminar pressure gradient, the steepness of which depends on the pressure difference and the lamina cribrosa thickness. The thin lamina cribrosa in the eyes of the glaucoma group versus the eyes of the normal group may therefore lead to a steepening of the translaminar pressure gradient which, among other factors, such as a change in the biomechanical tissue properties of the lamina cribrosa, may be responsible for the increased glaucoma susceptibility in eyes with advanced glaucoma. In a similar manner, we concluded that the thinning of the lamina cribrosa in glaucomatous eyes with a markedly elongated axial length may be one of the reasons that a markedly elongated length (and thus high myopia) is a risk factor for glaucomatous optic neuropathy.\(^11,21–26\) Fitting the hypothesis of the importance of the translaminar pressure difference and gradient, in a recent clinical study, Berdahl et al.\(^27\) suggested that patients with chronic open-angle glaucoma have a lower cerebrospinal fluid pressure than do normal subjects.

With respect to the peripapillary scleral thickness, the present study suggested that it decreases significantly with increasing axial length, independent of the presence of glaucoma. Since the peripapillary sclera may have a major impact on the biomechanics of the lamina cribrosa, as has already been pointed out by Downs et al.\(^1,28\) Bellezza et al.,\(^29\) Burgoyne and Morrison,\(^30\) Yang et al.,\(^3,5\) and Sigal et al.,\(^1\) the finding may suggest that along with a thinning of the lamina cribrosa, a thinning of the peripapillary sclera may play an additional role in the pathogenesis of increased glaucoma susceptibility in eyes with a markedly elongated axial length. The thickness of the peripapillary sclera did not vary significantly between the non-axially elongated glaucoma group and the normal group, suggesting that the glaucomatous process may not lead to a marked thinning of the peripapillary sclera (Fig. 6). Correspondingly, the multivariate analysis including all eyes of the study showed that the presence of glaucoma was not significantly associated with the thickness of the peripapillary sclera. It may suggest that the increase in glaucoma susceptibility with the stage of glaucoma in non-axially elongated eyes may not be mainly due to changes in the thickness of the peripapillary sclera. It was remarkable that approximately 50% of the posterior sclera contributed to the sclera close to the optic nerve scleral canal, whereas approximately 50% merged with the optic nerve meninges. It has remained unclear so far which of these parts or regions of the sclera are to what extent biomechanically important for stress and strain in the lamina cribrosa. Besides glaucoma, the progressive peripapillary scleral thinning with increasing axial length may have clinical and pathogenic importance for posterior staphylomata. The existence of scleral staphylomata which are a hallmark of extremely myopic eyes with extremely elongated axial length fits well and almost confirms the data of the present study in that eyes with a markedly elongated axial length have a thinner peripapillary sclera than non-axially elongated eyes. Since posterior staphylomata are clinically not found in non-axially elongated eyes with advanced glaucoma, they do also fit with the results of our study that the thinning of the peripapillary sclera was associated with markedly elevated axial length and that it is not associated with glaucoma.

The lengths of the inner and outer surface lines in the lamina cribrosa were significantly longer in the glaucoma group than in the normal group (Table 2). Since the length of the Bruch’s membrane opening did not vary significantly between both study groups, the parameters inner lamina cribrosa surface line length divided by the length of Bruch’s membrane opening and outer lamina cribrosa surface line length divided by the length of Bruch’s membrane opening were significantly larger in the glaucomatous group than in the normal group. The result suggests that the glaucoma related cupping in non-axially elongated eyes may be associated with an elongation or stretching of the lamina cribrosa leading to longer surface lines. The finding that the lamina cribrosa surface lines were longest in the glaucomatous elongated axial length group may be due to the additional enlargement of the optic disc related to the marked elongation of the globe.\(^31\) Correspondingly, the length of Bruch’s membrane opening was significantly longer in the glaucomatous elongated axial length group than in any of the two other study groups (Table 2).

There was generally a notable interindividual variability in the measurements of the lamina cribrosa and the thickness of the peripapillary sclera (Figs. 4, 6). This interindividual variability was partially due to limitations of the study techniques such as oblique histologic sectioning and variations in the locations from which the sections were taken. The interindividual variability in the measurements led to a marked overlapping between the study groups (Table 2, Figs. 4, 6). That the differences between the study groups were often higher than the standard deviations of the means, however, explains the statistical significance of the differences between the study groups for most of the parameters measured. The interindividual variations in the measurements can also be one of the reasons for the interindividually variable susceptibility for glaucomatous optic nerve damage.

The following limitations should be considered in interpreting our results: (1) The normal eyes were not normal
eyes, since they contained malignant choroidal melanoma; however, because the tumor cells neither invaded the optic nerve head nor the trabecular meshwork, it is unlikely that the presence of the malignant melanoma influenced the results of the study. (2) In our two glaucomatous groups, only eyes with end-stage glaucomatous damage were studied by what may be the most advantageous method to find differences between normal eyes and eyes with glaucoma. It may be considerably more difficult to find thinning of the lamina cribrosa early in a neuropathy. In fact, in two recent studies, the lamina cribrosa, although clearly posteriorly deformed, was actually thicker in perfusion-fixed monkey eyes with very early experimental glaucoma compared with the contralateral normal eyes.26-29 Conclusions drawn from the present study may therefore only be that thinning of the lamina cribrosa, perhaps in a sense of compression or collapse, is present in a relatively late stage of glaucomatous optic neuropathies. (3) The ages of our groups were different, with the normal group being significantly younger than the two glaucomatous groups. The differences in the outcome parameters between the study groups were highly significant, however, so that again it may be unlikely that the age difference between the study groups had a marked effect on the study results. In addition, the glaucoma group and the glaucomatous elongated axial length group did not vary in age. Furthermore, the lamina cribrosa thickness was not correlated with age in a multivariate analysis. (4) We measured only a single section per eye, and the section was not consistent among and between groups. Serial sections of the globes were not available so that it was not possible to determine whether the histologic section was located in the very center of the optic disc or ran paracentrally. Since there were no marked differences in the thinning of the lamina cribrosa between the central region versus the peripheral region of the optic disc, the inaccuracy in the exact localization of the histologic section may not have markedly influenced the determinations of the lamina cribrosa thickness and the conclusions of the study. In addition, this limitation in the design of the study again held true for all three study groups so that this systemic error may not have markedly influenced the results of the comparison of the study groups. In a similar manner, the histologic sections were not of a consistent orientation. The glaucomatous eyes were sectioned in a vertical direction, while the eyes of the nonglaucomatous control were sectioned according to the location of the tumor. Considering that the lamina is remarkably variable in its three-dimensional geometry, as shown by Bellezza et al.29 and Burgoyne and Morrison,30 the lamina cribrosa may be thinnest in different regions of the optic nerve head. Since the differences in the measurements between the three study groups were highly significant for all locations measured, it may be unlikely that this flaw in the study design markedly influenced the conclusions of the investigation. (5) Unlike in previous studies, we failed to determine a difference in peripapillary scleral thickness in the glaucoma group and may have missed regional differences. (6) The eyes examined in our study were fixed at an intraocular pressure of about 0 mm Hg. Previous studies on nonhuman primates by Burgoyne and Morrison have shown differences in the laminar and peripapillary scleral morphology between eyes fixed at physiologic pressures and at 0 mm Hg. This difference may have introduced an artifact into our study. As long as there were no marked differences in material properties, however, it may not have introduced a profound systemic bias and may not have influenced the results and conclusions of the study. (7) Because of swelling of the tissue after enucleation and due to the histologic preparation of the slides, the measurements given in this study will not represent dimensions as determined intravally. It was, however, not our purpose to evaluate the measurements of the lamina cribrosa and its surrounding tissues in real dimensions, but to compare the measurements of the lamina cribrosa obtained in markedly axially elongated eyes with measurements taken in non-axially elongated eyes, with or without glaucoma. The systemic error that was introduced by the histologic preparation of the slides will have affected the specimen of all three study groups in a similar manner, since the preparation did not vary between the study groups. It may thus not have markedly affected the conclusions of the study.

Another aspect to be taken into account is that the histomorphometric measurements obtained in the present study on Chinese eyes were generally markedly smaller than those obtained in the previous study of Caucasian eyes.9-11 The reasons for the discrepancies between the studies may be differences in the histologic preparation of the slides including fixation (4% formaldehyde in the present study versus a solution of 4% formaldehyde and 1% glutaraldehyde in the studies of Caucasian eyes) and staining as well as differences in the determination of the anterior surface and posterior border of the lamina cribrosa by the examiners. The differences between the studies may not indicate marked real differences between the two ethnic groups. Since it was not the purpose of the study to compare the lamina cribrosa measurements between Caucasian and Chinese eyes but to evaluate intraethnic differences between groups of Chinese eyes, a potential systemic error in the outlining of the lamina cribrosa may therefore not have markedly influenced the results and conclusions of the study.

It should be noted that our data on the relative thickness of the lamina cribrosa and peripapillary sclera in the glaucomatous group and the glaucomatous elongated axial length group may in fact be profoundly influenced by the age of the patients in each group, since we do not have data on axial length and refractive error before the onset of the ocular problems that led to their enucleation. In fact, our group of patients with a markedly elongated axial length may not have had high myopia before the onset of intraocular pressure elevation. The subset of patients who were myopic at the time of enucleation may have become so secondarily, as a response to chronic high intraocular pressure elevation. It means that it is possible that eyes may axially elongate under the influence of high intraocular pressure depending on scleral material properties. This elongation may occur also after the age of 2 to 4 years. One may assume that if the intraocular pressure is high for a long enough period (i.e., several years), the eyes of relatively young patients, such as those included in our study, may become somewhat elongated. There were no data on the magnitude of the exposure to high intraocular pressure, and there were no hard data on the age at onset. In contrast, the younger age of the patients with the markedly elongated (highly myopic) eyes in our study suggested an elongation of the globes at the onset of high intraocular pressure at a young age. This supposition may hold true particularly since the age at the known onset of the elevation of intraocular pressure was significantly lower in the glaucomatous elongated axial length group than in the non-axially elongated glaucoma group (Table 1). The finding that the corneal diameters did not vary between the highly elongated eyes and the nonelongated eyes does not absolutely contradict that the elongation in the highly myopic group was due to the high intraocular pressure. As long as the material properties of the cornea and sclera have not adequately been examined yet, one cannot exclude the possibility that the
high intraocular pressure led to a distension of the posterior sclera in the glaucomatous elongated axial length group whereas the cornea kept a normal size due to a difference in the material properties between the posterior sclera and the cornea. In addition, the ocular trauma that led to the high intraocular pressure was usually associated with a corneal scar that may have additionally stiffened the cornea.

Correspondingly, a large body of literature documents profound enlargement of the globe accompanied by thinning of the sclera in a variety of childhood glaucomas. The fact that the mean age of the glaucomatous elongated axial length group was 27.9 years compared with those of the normal and the glaucomatous groups suggests the possibility that a profound enlargement of the globe and thinning of the peripapillary sclera and lamina were part of the response to the chronic, high intraocular pressure elevation in all eyes, and that it reached the extreme in patients who were most susceptible (i.e., had the most compliant sclera and were exposed to the highest levels of intraocular pressure). In this scenario, young patients, with more compliant sclera, thinned most (becoming the highly myopic group on the basis of axial elongation of the globe) and older patients in which the sclera was presumably stiffer and therefore more resistant to deformation, axial elongation was less likely to occur, as was detectable thinning of the peripapillary sclera. Because of its retrospective nature of the study, these two interpretations of our data cannot be discounted.

In view of all the limitations mentioned, the present study as well as the previous histomorphometric investigations using the same methods have clearly to be considered preliminary pilot studies, until prospectively planned investigations of serial sections have been performed. In these future studies, the techniques used in the present investigation may no longer be adequate but may require fundamental changes.

In conclusion, the present study suggests that the lamina cribrosa and peripapillary sclera are thinnest in eyes with the longest axial length in Chinese eyes with secondary intraocular pressure elevation. The lamina cribrosa was thinnest and associated with a statistically significant thinner peripapillary sclera in those eyes that demonstrated postmortem axial lengths of greater than 27.5 mm. The lamina cribrosa was thinner, but the peripapillary sclera was not thinner in those glaucomatous eyes with an axial length of less than 27.5 mm. Because these data are retrospective, they may not directly pertain to eyes that are myopic in the setting of normal levels of intraocular pressure or eyes that are myopic before the onset of an intraocular pressure elevation.

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References


