Evaluation of scleral and corneal thickness in keratoconus patients

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PURPOSE: To determine whether the scleral stroma is affected as much as the corneal stroma in keratoconus.

SETTING: University Eye Clinic, Bern, Switzerland.

DESIGN: Comparative case-control study.

METHODS: Eyes with keratoconus (keratoconus group) and eyes of age-, sex-, and axial length–matched controls (control group) were analyzed. Corneal videokeratometry and pachymetry were performed using a Scheimpflug tomographer (Pentacam). For measurements of the peripheral cornea and the anterior sclera, a spectral-domain anterior segment optical coherence tomography device (Spectralis) was used.

RESULTS: The study group comprised 51 eyes and the control group, 50 eyes. The mean central corneal thickness in the keratoconus group was statistically significantly lower than in the control group (447.8 μm ± 57.8 [SD] versus 550.5 ± 35.5 μm) ($P < .0001$). No significant difference in the mean anterior scleral thickness was found between the keratoconus group and the control group (479.1 ± 43.7 μm versus 474.2 ± 43.0 μm) ($P = .57$).

CONCLUSION: Although corneal thinning was observed in keratoconus patients, the anterior scleral stroma thickness in these patients seemed to be similar to that in healthy control eyes.

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Keratoconus is a bilateral noninflammatory progressive disease of the cornea. It is characterized by thinning and ectasia of the cornea with subsequent irregular astigmatism and central scarring. With an incidence of approximately 50 to 230 per 100 000 people, keratoconus is the most frequent corneal dystrophy.[1,2]

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Keratoconus was first described by Nottingham in 1854.[3] Whereas corneal changes in keratoconus are well documented, the exact mechanisms leading to this sight-threatening disease are not yet known.[4] One focus of keratoconus research with recent therapeutic advances in the realm of corneal crosslinking has been on the composition of collagen in keratoconus patients.[5,6] The corneal stroma in the cone area of keratoconus patients has significant differences in collagen types XIII, XV, and XVIII.[7,8] Furthermore, certain types of connective tissue disorders, such as Marfan syndrome and Ehlers-Danlos syndrome, are associated with keratoconus, corroborating the role of collagen in keratoconus pathogenesis.[4,9,10]

Although it has been known for more than a century[11] that corneas of keratoconus patients show central corneal thinning, a recent study[12] suggests

that the thinning affects the corneal periphery as well. Given the thinning of the cornea at the limbus and the postulated relationship between the disease pathogenesis and collagen composition in the area of thinning, it might be speculated that the sclera is involved with the keratoconus disease process as well. In recent years, optical coherence tomography (OCT) instruments such as spectral-domain anterior segment OCT (AS-OCT) devices have allowed noninvasive measurements not only of the peripheral cornea but also of the anterior sclera. In addition, modern keratometry systems such as the Pentacam (Carl Zeiss Meditec AG), which uses a rotating Scheimpflug camera to generate elevation topography and aberration maps, allow analysis of the peripheral cornea.[13]

The goal of this study was to evaluate the peripheral cornea and the anterior sclera in keratoconus patients.

PATIENTS AND METHODS

This observational case-control study comprised subjects recruited from the Department of Ophthalmology at Bern University Hospital, Bern, Switzerland, between July 2013 and April 2014. The aim was to assess scleral thickness in patients with keratoconus using enhanced-depth AS-OCT. The study was approved by the local ethics committee in Bern. All keratoconus patients (keratoconus group) and control subjects (control group) provided written informed consent.

Patients in the keratoconus group had a comprehensive ophthalmic examination including assessment of corrected distance visual acuity, slitlamp biomicroscopy followed by corneal pachymetry and tomography with the Pentacam rotating Scheimpflug camera, and imaging of the anterior sclera using a spectral-domain OCT device (Spectralis OCT) with an anterior segment module (Heidelberg Engineering, Inc.).

Inclusion criteria for the keratoconus group were eyes with established keratoconus, defined as the presence of at least 2 of the following clinical signs: scissoring seen on retinoscopy, central or paracentral corneal thinning with ectasia, anterior corneal scarring, hemosiderin deposition (Fleischer ring), posterior stromal striae (Vogt striae), or anterior bulging of the cornea (Munson sign). The exclusion criterion was previous perforating keratoplasty.

In addition to the clinical assessment, differentiation between keratoconus patients and healthy controls was made using the D value, which was obtained from the rotating Scheimpflug camera system. The D value is based on pachymetry and elevation using a linear regression analysis and represents a keratoconus index that aims to detect early ectatic disease.[14] A final D value of 1.6 standard deviation (SD; SD from the mean) or more is suspicious, and a D value of 2.6 SD or more is pathological. Therefore, in addition to the clinical criteria, subjects with a D value of 2.6 SD or more were considered keratoconus patients (Figure 1, *A* and *B*).

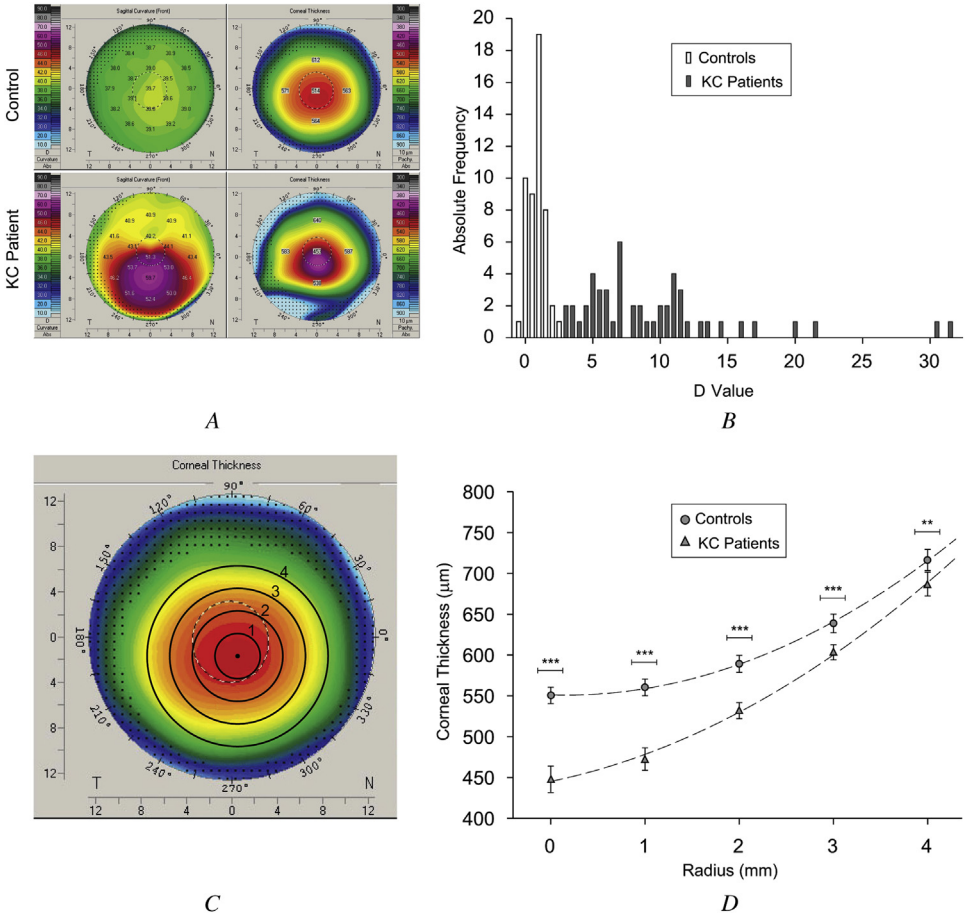

Figure 1. Separation of the control patients and keratoconus patients. *A*: Rotating Scheimpflug camera analysis of the right eye of a healthy control patient and of a keratoconus patient; the left column shows the sagittal curvature of the corneal front, and the right column shows the corneal thickness. *B*: Distribution of the D values in the study population (N = 101 eyes). *C*: Representative rotating Scheimpflug camera analysis of a right eye showing the measurement locations. Corneal rings with radii of 1.0 mm, 2.0 mm, 3.0 mm, and 4.0 mm are located around the thinnest location (0.0 mm). *D*: Mean corneal thickness from the thinnest location to the peripheral cornea (*** = $P < .001$, 1-way ANOVA; ** = $P < .01$, 1-way ANOVA; KC = keratoconus).

In the rotating Scheimpflug camera analysis, the central cornea (Figure 1, *C*) was defined as 0.0 mm in both groups. Based on this central point, rings with radii of 1.0 mm, 2.0 mm, 3.0 mm, and 4.0 mm were defined, and the corneal thickness was averaged along these rings. Furthermore, the temporal, inferior, nasal, and superior corneal thicknesses were measured on a ring with a radius of 1.5 mm around the pupil center (Figure 2, *B*).

In addition to a pathological D value, any history or suspicion of keratoconus and/or a corneal thickness less than 490 µm (measured at the thinnest location) or anterior segment pathology likely to interfere with scleral thickness measurements were exclusion criteria for the control group. For OCT imaging, the spectral-domain AS-OCT device was used and the sclera mode with enhanced-depth imaging was chosen with an OCT grid of 21 lines × 21 lines each measuring 8.1 mm. The spectral-domain AS-OCT photographs were taken with a resolution of 768 × 496 (381 KB) and a scan angle of 15 degrees. Using Heidelberg Eye Explorer software (version 1.7.1.0, Heidelberg Engineering, Inc.), a 2.0 mm distance from the scleral spur was measured in the superotemporal (S–T), inferotemporal (I–T), superonasal (S–N), and inferonasal (I–N) quadrants (Figure 3, *A*). The sclera proper was discerned from the conjunctiva and the episclera by identifying the deep episcleral vascular plexus (Figure 3, *B*). In each quadrant, at least 4 OCT scans were acquired, from which 3 with the best image quality were chosen. (Each line in Figure 3, *A*, corresponds to an OCT scan.) The 3 measurements were averaged for each quadrant for further analysis. Spectral-domain AS-OCT images were measured by 3 masked observers (B.S., M.B., C.T.) to assess the measurement accuracy. The axial length (AL) was measured using partial coherence interferometry (IOLMaster, Carl Zeiss Meditec AG).

Results are presented as the mean ± SD. After normal distribution was confirmed using the D'Agostino and Pearson omnibus normality test, analysis between the groups was

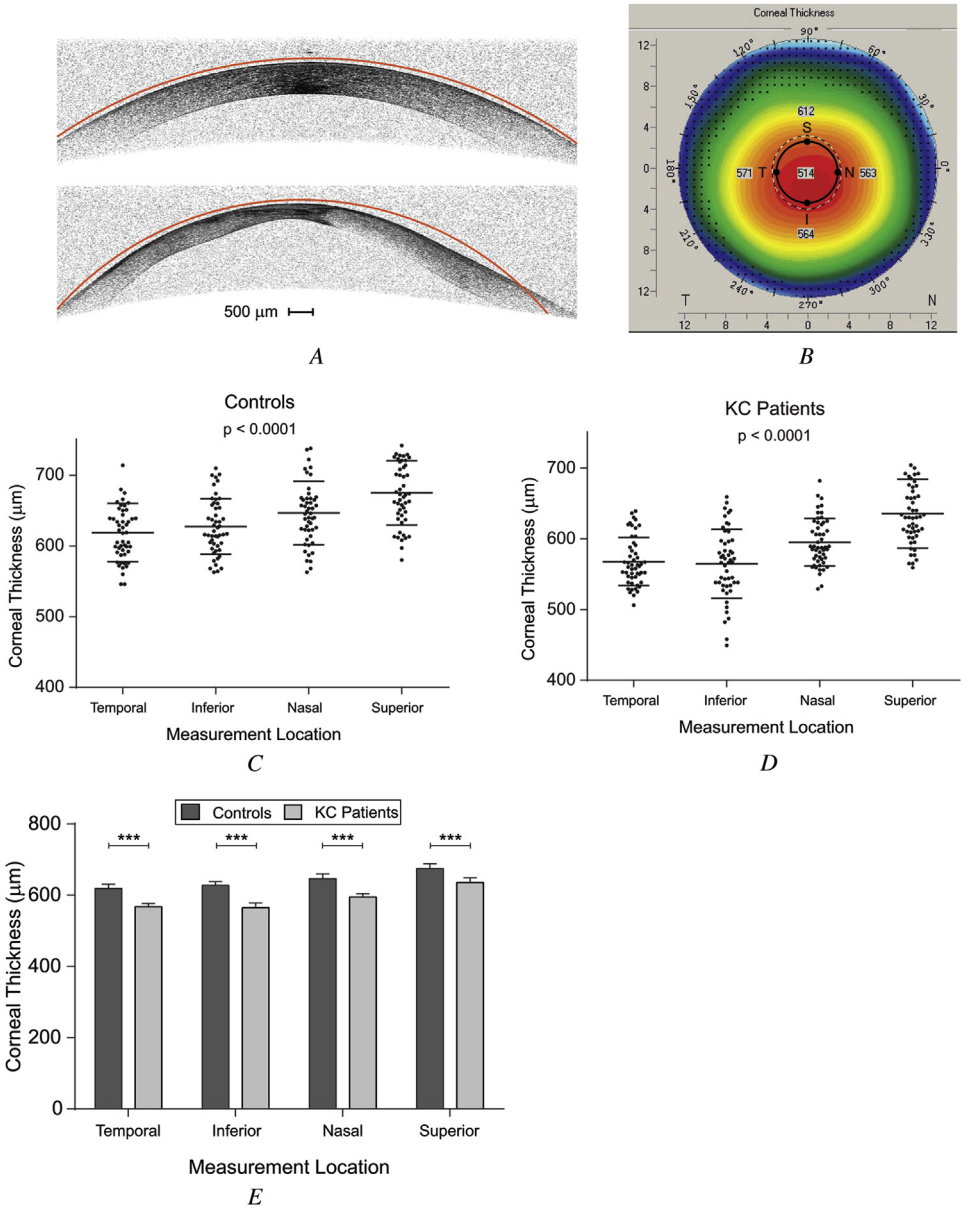

Figure 2. Corneal pachymetry using the rotating Scheimpflug camera. *A*: Representative AS-OCT image of a healthy (*top*) and a keratoconic (*bottom*) cornea. The red arcs illustrate the approximately spherical cornea of the healthy subject and the irregular cornea of the keratoconus (KC) patient. *B*: Representative rotating Scheimpflug camera image of a right eye illustrating the measurement locations (I = inferior quadrant; N = nasal quadrant; S = superior quadrant; T = temporal quadrant) located on a ring (radius 1.5 mm) around the pupil center. *C*: Dot plot showing corneal pachymetry (mean ± SD) of controls measured in the temporal, inferior, nasal, and superior quadrants (n = 50) ($P < .0001$, 1-way ANOVA). *D*: Dot plot showing corneal pachymetry (mean ± SD) of keratoconus patients (n = 51) ($P < .0001$, 1-way ANOVA). *E*: Comparison of the mean central corneal thickness (mean ± 95% confidence interval) of control subjects and keratoconus patients (N = 101) (*** = $P < .001$, unpaired *t* test).

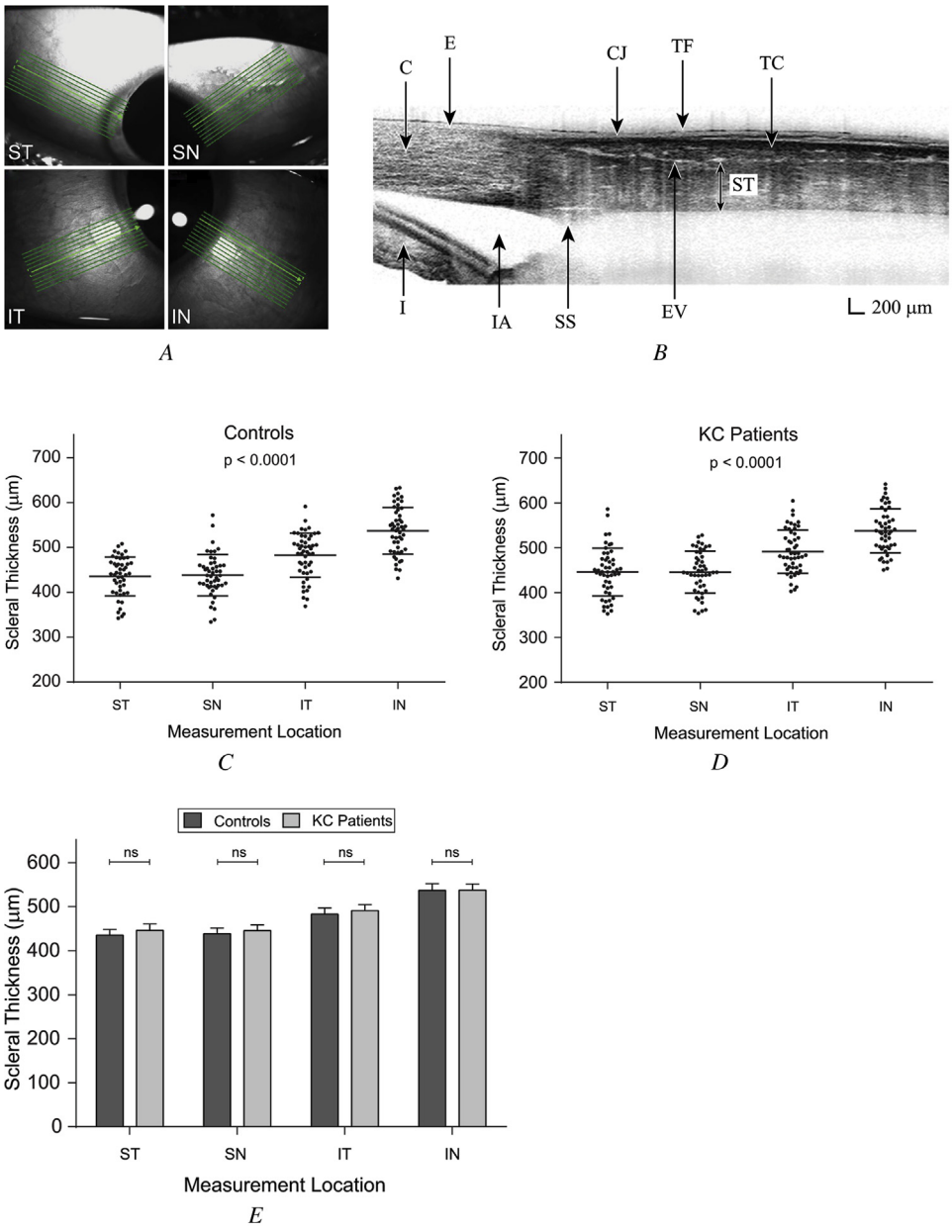

Figure 3. Scleral pachymetry. *A*: Infrared images showing the location of scleral measurements (IN = inferonasal quadrant; IT = inferotemporal quadrant; SN = superonasal quadrant; ST = superotemporal quadrant). Each green line corresponds to an OCT scan. *B*: Representative AS-OCT image of the sclera (C = cornea; CJ = conjunctiva; E = corneal epithelium; EV = episcleral vessels; I = iris; IA = iridocorneal angle; SS = scleral spur; ST = scleral thickness; TC = Tenon capsule; TF = tear film). *C*: Dot plot of scleral thickness measurements (mean ± SD) of controls measured in 4 quadrants (n = 50) ($P < .0001$, 1-way ANOVA). *D*: Dot plots of scleral thickness (mean ± SD) of keratoconus (KC) patients (n = 51) ($P < .0001$, 1-way ANOVA). *E*: Comparison of the mean scleral thickness (mean ± 95% confidence interval) of the control subjects and keratoconus patients (n = 101) (ns = not significant, unpaired *t* test).

performed using the unpaired *t* test or 1-way analysis of variance (ANOVA). The level of significance was 0.05 (2 sided) in all statistical tests. The Spearman correlation (*r*) was applied for the correlation of Scheimpflug pachymetry measurements with corneal thickness measurements using AS-OCT, and results were displayed as Bland-Altman plots. Statistical analysis was performed using Prism software (version 6, Graphpad Software, Inc.).

RESULTS

The study included 55 subjects recruited between July 2013 and April 2014. The keratoconus group comprised 51 eyes of 29 patients (7 women, 22 men) and the control group, 50 eyes of 26 patients (6 women, 20 men). The mean age was 30.8 ± 8.5 years (range 18 to 45 years) and 30.7 ± 6.3 years (range 22 to 49 years), respectively. The keratoconus group and control group were comparable in sex distribution and mean age (both $P > .05$). In the keratoconus group, 7 single eyes were excluded from the study, 4 of them for having had keratoplasty and 3 for having a normal D value, as described above. Two eyes were excluded in the control group because of the presence of conjunctivitis and keratitis. Table 1 shows the AL measurements, maximum simulated keratometry (K) reading, and corneal and scleral thicknesses.

Corneal Pachymetry

Measuring the corneal thickness at distances from the thinnest location using a 1.0 mm, 2.0 mm,

Table 1. Axial length measurements, maximum simulated K reading, and corneal and scleral thicknesses by group.

Parameter	Mean ± SD		*P* Value*
	KC Group	Control Group	
AL, mm	24.48 ± 0.83	23.17 ± 1.45	.3
K_{max} (D)	55.4 ± 7.5	44.2 ± 1.8	<.0001
Corneal thickness (µm)†	447.8 ± 57.8	550.5 ± 35.5	<.0001
Scleral thickness (µm)‡	479.1 ± 43.7	474.2 ± 43.0	.5730

AL = axial length; KC = keratoconus; K_{max} = maximum simulated keratometry reading
*Unpaired *t* test (N = 101 eyes)
†At the thinnest location
‡Average of inferonasal, inferotemporal, superotemporal, superonasal

Table 2. Corneal pachymetry.

Parameter	Mean (µm) ± SD		*P* Value*
	KC Group	Control Group	
Thinnest location	447.8 ± 57.8	550.5 ± 35.5	<.0001
Ring at 1.0 mm	472.6 ± 49.2	560.3 ± 35.8	<.0001
Ring at 2.0 mm	531.9 ± 35.4	589.2 ± 37.0	<.0001
Ring at 3.0 mm	603.4 ± 32.9	638.7 ± 40.1	<.0001
Ring at 4.0 mm	687.0 ± 50.4	716.4 ± 45.6	.0031
Temporal	567.7 ± 34.1	619.0 ± 41.1	<.0001
Inferior	564.7 ± 48.6	627.4 ± 39.2	<.0001
Nasal	594.9 ± 33.6	646.7 ± 45.0	<.0001
Superior	635.4 ± 48.7	675.1 ± 45.4	<.0001

KC = keratoconus
*Unpaired *t* test (N = 101 eyes)

3.0 mm, and 4.0 mm circle showed that even though the thinning was greatest at the central cornea in the keratoconus group compared with the control group, the peripheral cornea was also significantly thinner in the keratoconus group. The results are shown in Table 2 and Figure 1, *D*.

Corneal pachymetry measured temporally, inferiorly, nasally, and superiorly 1.5 mm from the pupil center showed significantly thicker corneal measurements in the superior quadrant followed by the nasal and inferior quadrants (P <.0001, 1-way ANOVA) (Figure 2, *C* and *D*). The results are shown in Table 2.

The mean corneal thickness in each quadrant was significantly thinner in the keratoconus group than in the control group (Figure 2, *E*).

Scleral Thickness Measurements: Enhanced-Depth Spectral-Domain Anterior-Segment Optical Coherence Tomography

The scleral thickness in the keratoconus group increased from the S–T quadrant to the S–N quadrant to the I–T quadrant to the I–N quadrant (Figure 3, *D*). The control group had the same distribution (Figure 3, *C*). Overall, there was no significant difference in scleral thickness between the keratoconus group and the control group (Figure 3, *E*). Table 3 shows the results.

Factors Influencing Scleral Thickness Measurements

The range of AL in both the keratoconus group and the control group was 20.9 to 27.2 mm; there was a positive correlation between the AL and the scleral thickness (r = 0.2518, P =.01) (Figure 4, *B*). In addition, there was a strong dependency between sex and scleral thickness measurements, with women having statistically significantly decreased mean anterior scleral thickness (P < .05) (Figure 4, *A*).

Comparison Between Scheimpflug and Spectral-Domain Anterior Segment Optical Coherence Tomography

There was a good correlation between the 2 methods in both groups, as shown in the Bland-Altman plots for agreement of the peripheral corneal measurement between Scheimpflug corneal pachymetry at the 4.0 mm ring and spectral-domain AS-OCT (Figure 4, *C1* and *C2*). The corneal thickness measurements by OCT were performed approximately 1.0 mm from the scleral spur and showed a good correlation with the pachymetry values from the rotating Scheimpflug camera (r = 0.61, P <.0001). Nevertheless, the Bland-Altman plots show a tendency for the OCT method to yield thicker peripheral corneal measurements than the rotating Scheimpflug camera, with a mean difference of approximately 45 µm (Figure 4, *C1* and *C2*).

DISCUSSION

Recent evidence has shown that in keratoconus, corneal thinning is also present outside the cone

Table 3. Scleral pachymetry.

Parameter	Mean (µm) ± SD		*P* Value*
	KC Group	Control Group	
Superotemporal	445.8 ± 53.1	435.4 ± 43.3	.2893
Superonasal	445.3 ± 46.7	438.5 ± 46.0	.4717
Inferotemporal	491.1 ± 48.1	482.7 ± 49.3	.3870
Inferonasal	537.5 ± 48.9	537.2 ± 51.9	.9745

KC = keratoconus
*Unpaired *t* test (N = 101 eyes)

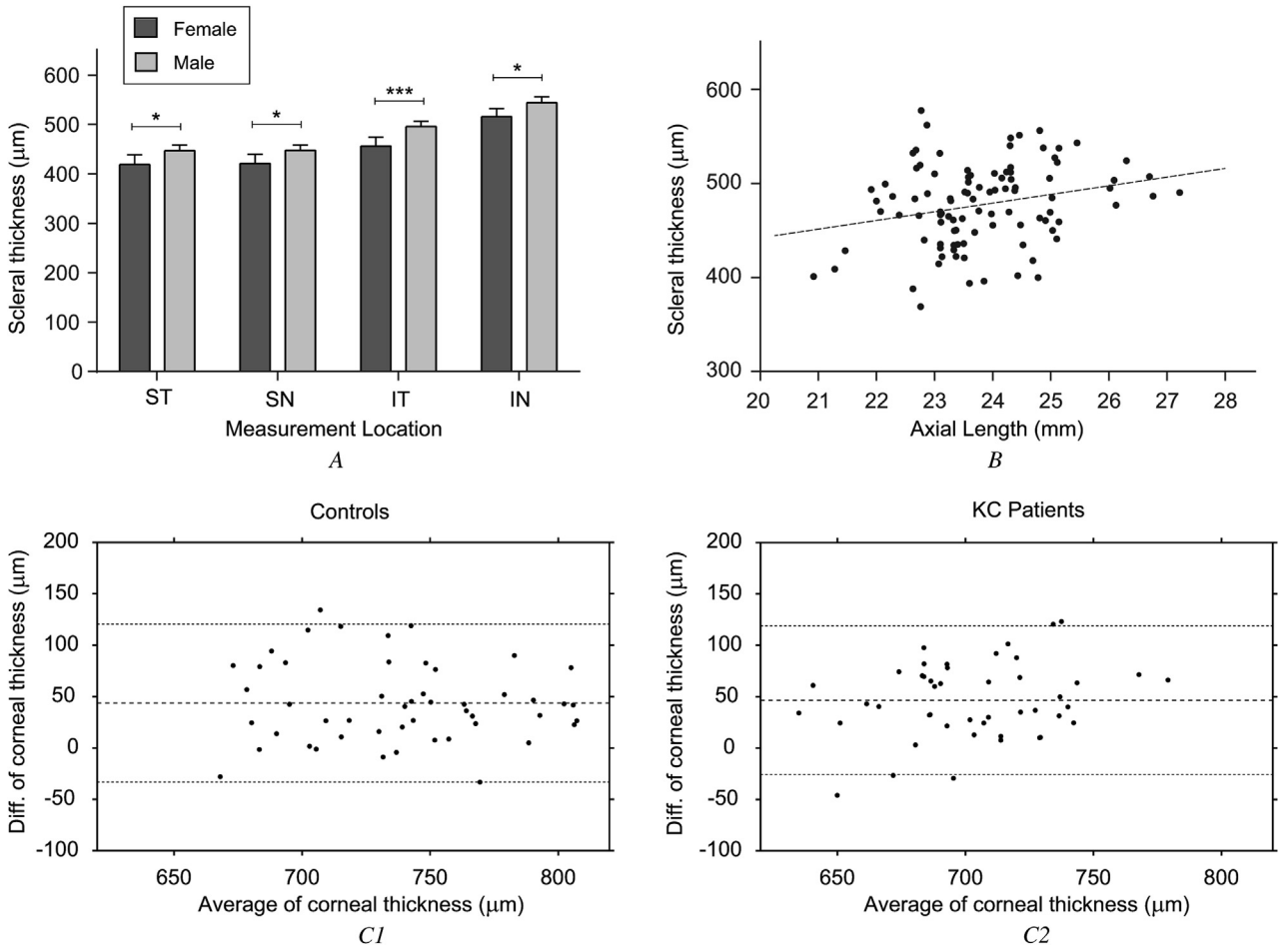

Figure 4. Scleral thickness influenced by sex and by AL. *A*: Scleral thickness measurements (mean ± 95% confidence interval) in individual quadrants for women and men in the entire study population (N = 101) (*= $P < .05$, unpaired *t* test; *** = $P < .001$, unpaired *t* test; IN = inferonasal quadrant; IT = inferotemporal quadrant; SN = superonasal quadrant; ST = superotemporal quadrant). *B*: Scatterplot with linear regression showing the correlation of AL with scleral thickness (n = 99) ($r = 0.2518$, $P = .01$). Scleral thickness is the average of the S–T, S–N, I–T, and I–N scleral thickness. *C*: Agreement of peripheral corneal pachymetry by rotating Scheimpflug camera and AS-OCT. *C1*: Bland-Altman plot showing the agreement of the 2 methods for control subjects (n = 50) ($r = 0.61$, $P < .0001$) (Diff. = difference). *C2*: Bland-Altman plot showing the agreement of the 2 methods in keratoconus (KC) patients (n = 46) ($r = 0.50$, $P = .0003$). Dotted lines define the 95% confidence interval. Rotating Scheimpflug camera measurements were taken at the 4.0 mm ring.

area up to the limbus.[12] Despite fundamentally different optical properties of these 2 tissues, resulting mainly from the orientation of collagen fibrils,[15] both the cornea and the sclera originate embryonically from the mesenchyme[16] and have comparable collagen content.[15] Therefore, it might be speculated that changes similar to thinning might also occur in the sclera of eyes with keratoconus. In the cornea and the sclera, the major collagen component is type I, representing approximately 70% of the dry weight in each.[17,18] In addition, fibrils consisting of types III, V, VI, and XII are present in both the cornea and the sclera.[15,19,20] To our knowledge, the present study is the first to compare the anterior scleral thickness in patients with diagnosed keratoconus with that in an age-matched and sex-matched healthy control group.

In addition to a strong genetic component,[21,22] several biochemical and biomechanical factors, such as chronic eye rubbing, contact lens wear, and atopy, have been proposed as causes of disease development in keratoconus patients.[4] Histopathologically, a variation in the central and peripheral epithelial thickness has been found. Furthermore, a majority of patients showed marked thickening of the epithelial basement membrane whereas the anterior limiting lamina was thinned or lost, mainly in the area of the cone. However, in a study examining 12 keratoconus corneas,[23] the peripheral cornea was affected as well, although to a lesser extent.

Given the association of keratoconus with systemic connective tissue diseases such as Ehlers-Danlos syndrome[4] or Marfan syndrome, it has been suggested that the composition of collagen plays a role in keratoconus.[10] Marfan syndrome is caused by mutations in the *FBN1* gene (MIM *134797), leading to alterations in extracellular matrix protein fibrillin-1.[24] Ehlers-Danlos syndrome is caused by mutations in *COL3A1* (MIM *120180), which encodes the pro-α1 chains of type III collagen, a fibrillar collagen that is found in connective tissues frequently in association with type I collagen.[25] Despite clinical observations such as blue sclera in Ehlers-Danlos syndrome,[26] there has been a lack of information regarding the scleral architecture and thickness in patients with connective tissue disorders.

Two findings in our study merit further discussion. We did not find any sign of axial elongation in our keratoconus group compared with the control group. This is in accordance with previous reports.[27] Despite the limited range of AL measurements, our study showed a weak positive correlation of AL with scleral thickness. Although this might at first seem counterintuitive, it does not contradict previous studies in which scleral thickness anterior to the equator was not positively correlated with AL.[28] The other finding is the strong sex dependency of scleral thickness measurements. To our knowledge, this has not been reported in the literature. Both findings have general implications for further studies of scleral thickness measurements.

To compare corneal pachymetry values obtained with OCT and the Pentacam rotating Scheimpflug camera, we quantified a region present on both imaging modalities; that is, the peripheral cornea. Although there was a good correlation between the rotating Scheimpflug camera and Spectralis spectral-domain AS-OCT measurements, it is obvious from the Bland-Altman plots that there was a tendency for the OCT method to yield thicker peripheral corneal measurements than the rotating Scheimpflug camera. We were able to show that spectral-domain AS-OCT measurements are useful to quantify scleral thickness in healthy subjects and those with keratoconus and that scleral thickness is highly variable between individual quadrants.

Our study has limitations, mainly the relatively small sample. Furthermore, the variation in disease severity in the keratoconus patients, represented by the broad distribution of D values, caused an inhomogeneous study population in that group.

Our findings suggest that keratoconus leads to thinning of the central and peripheral cornea but does not affect the thickness of the anterior scleral stroma. Further histological studies are necessary to conclusively exclude microstructural alterations of scleral collagen in patients with keratoconus.

WHAT WAS KNOWN

- Keratoconus leads to corneal thinning in the central cornea but affects the peripheral cornea as well.

WHAT THIS PAPER ADDS

- Keratoconus did not affect anterior scleral thickness.
- Scleral thickness values were influenced by AL and sex.

REFERENCES

1. Kennedy RH, Bourne WM, Dyer JA. A 48-year clinical and epidemiologic study of keratoconus. Am J Ophthalmol 1986; 101:267–273
2. Rabinowitz YS. Keratoconus. Surv Ophthalmol 1998; 42:297–319. Available at: http://www.keratoconus.com/resources/Major+Review-Keratoconus.pdf. Accessed January 23, 2015
3. Nottingham J. Practical Observations on Conical Cornea, and on the Short Sight, and Other Defects of Vision Connected With It. London, UK, John Churchill, 1854
4. Romero-Jiménez M, Santodomingo-Rubido J, Wolffsohn JS. Keratoconus: a review. Cont Lens Anterior Eye 2010; 33:157–166
5. Wollensak G, Spoerl E, Wilsch M, Seiler T. Keratocyte apoptosis after corneal collagen cross-linking using riboflavin/UVA treatment. Cornea 2004; 23:43–49
6. Spoerl E, Mrochen M, Sliney D, Trokel S, Seiler T. Safety of UVA–riboflavin cross-linking of the cornea. Cornea 2007; 26:385–389
7. Määttä M, Väisänen T, Väisänen M-R, Pihlajaniemi T, Tervo T. Altered expression of type XIII collagen in keratoconus and scarred human cornea; increased expression in scarred cornea is associated with myofibroblast transformation. Cornea 2006; 25:448–453
8. Määttä M, Heljasvaara R, Sormunen R, Pihlajaniemi T, Autio-Harmainen H, Tervo T. Differential expression of collagen types XVIII/endostatin and XV in normal, keratoconus, and scarred human corneas. Cornea 2006; 25:341–349
9. Babel J, Houber JP. Keratocone et sclerotiques bleues dans une anomalie congenitale du tissu conjonctif [Keratoconus and blue sclera in a congenital anomaly of connective tissue]. J Genet Hum 1969; 17:241–246
10. Maumenee IH. The cornea in connective tissue diseases. Ophthalmology 1978; 85:1014–1017
11. Gerlach J. Handbuch der allgemeinen und speziellen Gewebelehre des menschlichen Körpers, für Aerzte und Studierende. Mainz, Germany, Verlag von Eduard Janitsch, 1854
12. Brautaset RL, Nilsson M, Miller WL, Leach NE, Tukler JH, Bergmanson JPG. Central and peripheral corneal thinning in keratoconus. Cornea 2013; 32:257–261
13. Uçakhan ÖÖ, Özkan M, Kanpolat A. Corneal thickness measurements in normal and keratoconic eyes: Pentacam comprehensive eye scanner versus noncontact specular microscopy and ultrasound pachymetry. J Cataract Refract Surg 2006; 32:970–977. Available at: http://www.oculus.de/en/downloads/dyn/sonstige/sonstige/studies_oezelenen_turkey.pdf. Accessed January 23, 2015
14. Correia FF, Ramos I, Lopes B, Salomão MQ, Luz A, Correa RO, Belin MW, Ambrósio R Jr. Topometric and tomographic indices for the diagnosis of keratoconus. Int J Keratoconus Ectatic Corneal Dis 2012; 1:92–99. Available at: http://www.jaypeejournals.com/eJournals/ShowText.aspx?ID=3335&Type=FREE&TYP=TO

P&IN=~/eJournals/images/JPLOGO.gif&IID=258&isPDF=YES. Accessed January 23, 2015
15. Meek KM, Fullwood NJ. Corneal and scleral collagens—a microscopist's perspective. Micron 2001; 32:261–272
16. Hoar RM. Embryology of the eye. Environ Health Perspect 1982; 44:31–34. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1568953/pdf/envhper00461-0037.pdf. Accessed January 23, 2015
17. Keeley FW, Morin JD, Vesely S. Characterization of collagen from normal human sclera. Exp Eye Res 1984; 39:533–542
18. Maurice DM. The structure and transparency of the cornea. J Physiol 1957; 136:263–286. Available at: http://jp.physoc.org/content/136/2/263.full.pdf. Accessed January 23, 2015
19. Anderson S, SundarRaj S, Fite D, Wessel H, SundarRaj N. Developmentally regulated appearance of spliced variants of type XII collagen in the cornea. Invest Ophthalmol Vis Sci 2000; 41:55–63. Available at: http://www.iovs.org/content/41/1/55.full.pdf. Accessed January 23, 2015
20. Segev F, Héon E, Cole WG, Wenstrup RJ, Young F, Slomovic AR, Rootman DS, Whitaker-Menezes D, Chervoneva I, Birk DE. Structural abnormalities of the cornea and lid resulting from collagen V mutations. Invest Ophthalmol Vis Sci 2006; 47:565–573. Available at: http://www.iovs.org/content/47/2/565.full.pdf. Accessed January 23, 2015
21. Li X, Bykhovskaya Y, Caiado Canedo AL, Haritunians T, Siscovick D, Aldave AJ, Szczotka-Flynn L, Iyengar SK, Rotter JI, Taylor KD, Rabinowitz YS. Genetic association of COL5A1 variants in keratoconus patients suggests a complex connection between corneal thinning and keratoconus. Invest Ophthalmol Vis Sci 2013; 54:2696–2704. Available at: http://www.iovs.org/content/54/4/2696.full.pdf. Accessed January 23, 2015
22. Lu Y, Vitart V, Burdon KP, Khor CC, Bykhovskaya Y, Mirshahi A, Hewitt AW, Koehn D, Hysi PG, Ramdas WD, Zeller T, Vithana EN, Cornes BK, Tay W-T, Tai ES, Cheng C-Y, Liu J, Foo J-N, Saw SM, Thorleifsson G, Stefansson K, Dimasi DP, Mills RA, Mountain J, Ang W, Hoehn R, Verhoeven VJM, Grus F, Wolfs R, Castagne R, Lackner KJ, Springelkamp H, Yang J, Jonasson F, Leung DYL, Chen LJ, Tham CCY, Rudan I, Vatavuk Z, Hayward C, Gibson J, Cree AJ, MacLeod A, Ennis S, Polasek O, Campbell H, Wilson JF, Viswanathan AC, Fleck B, Li X, Siscovick D, Taylor KD, Rotter JI, Yazar S, Ulmer M, Li J, Yaspan BL, Ozel AB, Richards JE, Moroi SE, Haines JL, Kang JH, Pasquale LR, Allingham RR, Ashley-Koch A; NEIGHBOR Consortium, Mitchell P, Wang JJ, Wright AF, Pennell C, Spector TD, Young TL, Klaver CCW, Martin NG, Montgomery GW, Anderson MG, Aung T, Willoughby CE, Wiggs JL, Pang CP, Thorsteinsdottir U, Lotery AJ, Hammond CJ, van Duijn CM, Hauser MA, Rabinowitz YS, Pfeiffer N, Mackey DA, Craig JE, Macgregor S, Wong TY. Genome-wide association analyses identify multiple loci associated with central corneal thickness and keratoconus. Nat Genet 2013; 45:155–163. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3720123/pdf/nihms479022.pdf. Accessed January 23, 2015
23. Mathew JH, Goosey JD, Bergmanson JPG. Quantified histopathology of the keratoconic cornea. Optom Vis Sci 2011; 88:988–997. Available at: http://journals.lww.com/optvissci/Fulltext/2011/08000/Quantified_Histopathology_of_the_Keratoconic.14.aspx. Accessed January 23, 2015
24. Dietz HC, Cutting GR, Pyeritz RE, Maslen CL, Sakai LY, Corson GM, Puffenberger EG, Hamosh A, Nanthakumar EJ, Curristin SM, Stetten G, Meyers DA, Francomano CA. Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene [letter]. Nature 1991; 352:337–339
25. Pope FM, Martin GR, Lichtenstein JR, Penttinen R, Gerson B, Rowe DW, McKusick VA. Patients with Ehlers-Danlos syndrome type IV lack type III collagen. Proc Natl Acad Sci USA 1975; 72:1314–1316. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC432523/pdf/pnas00047-0100.pdf. Accessed January 23, 2015
26. Greenfield G, Romano A, Stein R, Goodman RM. Blue sclerae and keratoconus: key features of a distinct heritable disorder of connective tissue. Clin Genet 1973; 4:8–16
27. Lanier JD, Bullington RH Jr, Prager TC. Axial length in keratoconus. Cornea 1992; 11:250–254
28. Vurgese S, Panda-Jonas S, Jonas JB. Scleral thickness in human eyes. PLoS One 2012; 7(1):e29692. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3253100/pdf/pone.0029692.pdf. Accessed January 23, 2015