Endothelial alterations in 712 keratoconus patients – A report of the Homburger Keratoconus Center HKC

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Key words: corneal endothelium, keratoconus, specular microscopy, EM-3000, contact lens

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Word count: 4933

Abstract:

Purpose: To investigate the effect of keratoconus on the corneal endothelium using specular microscopy.

Methods: Seven hundred and twelve eyes from the Homburg Keratoconus Center (HKC) database were included in this retrospective study. Corneal endothelium was evaluated using the Tomey EM-3000 specular microscope. Keratoconus related topographic and tomographic data were obtained from Scheimpflug based tomography (Oculus Pentacam® HR). Eyes were classified into stages 0 (healthy) to 4 (severe keratoconus) according to the Topographic Keratoconus Classification (TKC). Subgroups were analyzed based on contact lens (CL) type (none/rigid/soft).

Results: The frequencies of keratoconus stages 0/1/2/3/4 according to TKC were 169/94/206/166/77. The endothelial cell density (CD) for the endothelial cell area for TKC 0/1/2/3/4 was 2611/2624/2557/2487/2401 cells per mm² and the coefficient of variation (CV) was 40.9/40.0/41.6/46.2/49.0%, respectively. The more severe the keratoconus stage the lower the endothelial cell count ($p<0.001$) and the higher the coefficient of variation ($p<0.001$). No contact lens wearing was noted in 207 eyes (NoCL), rigid CL in 200 (RCL) and soft CL in 54 (SCL). CD for NoCL/RCL/SCL was 2523/2533/2644 per mm² and CV was 41.8/54.1/43.1%, respectively. A significant difference in CV was found between NoCL and RCL ($p=0.02$), no significant difference in CV between NoCL and SCL ($p=0.07$). Endothelial cell density did not differ significantly between NoCL and RCL or SCL.

Conclusion: Endothelial cell density decreases and coefficient of variation increases significantly with increasing tomographic severity of keratoconus. In patients with rigid contact lenses compared to eyes without contact lens wear, we found a statistically significantly higher coefficient of variation of the endothelial cell size.

Introduction

Keratoconus is an ectatic non-inflammatory corneal disorder, in which the cornea forms a conic shape due to thinning of the corneal stroma (Rabinowitz 1998, Goebels et al. 2015). These changes normally induce irregular astigmatism, myopia and protrusion, leading to a mild to marked deterioration of visual performance (Rabinowitz 1998, Goebels et al. 2015) and loss of quality of life (Kymes et al. 2008). Keratoconus is usually bilateral, mostly diagnosed in the 2nd or 3rd decade of life with an annual incidence of 2 per 100.000 a year (Wagner et al. 2008) and a prevalence of 55:100.000 (Bühren et al. 2011, Nielsen et al. 2013, Goebels et al. 2015) and more prominent in males than in females. The last resort in therapy for severe keratoconus is still keratoplasty, either penetrating keratoplasty (PKP) or deep anterior lamellar keratoplasty (DALK) (El-Agha et al. 2014) with the benefit of avoiding endothelial immune reactions. For DALK the endothelial cell function of the host is the main determinant for the survival of the graft (El-Agha et al. 2014). In corneal buttons removed from PKP for keratoconus, histopathological changes of the endothelium such as pleomorphism and polymegalism have been found, as well as endothelial cell degeneration (El-Agha et al. 2014, Sturbaum & Pfeiffer 1993).

Several studies in the literature have investigated changes in corneal endothelium in eyes with keratoconus. Analysis of the endothelial cell layer was based either on specular or confocal microscopy. However, these studies provided non-uniform results. Eight different studies found either increased (Hollingsworth et al. 2005), decreased (Ucakhan et al. 2006, Niederer et al. 2008, Mocan et al 2008) or normal (El-Agha et al. 2014, Weed et al. 2007, Yeniad et al. 2010, Timucin et al. 2013) endothelial cell densities (ECD) in keratoconus patients. The sample sizes of these studies ranged between 29 and 68 cases.

The purpose of the present study was to investigate, whether the grade of keratoconus and/or the use of contact lenses affect corneal endothelium in a large study population of 712 eyes from our Homburger Keratoconus Center HKC.

Patients and Methods

This retrospective cross-sectional study was conducted at the Department of Ophthalmology, Saarland University Medical Center in Homburg/Saar, Germany. The study was approved by the local Ethics Committee (Ethik-Kommission der Ärztekammer des Saarlandes, Nr. 157/10) and followed the tenets of the Declaration of Helsinki. Informed written consent was obtained from all patients.

Patients:

Seven hundred and twelve eyes from the database of our Homburg Keratoconus Center (HKC) were included in this study. The aims of the HKC include research in diagnostics, longitudinal course of keratoconus, therapy options and influencing factors, such as the effect of thyroid gland function on keratoconus (Gatzioufas et al. 2008, Gatzioufas et al. 2014, Thanos et al. 2016) and have been described in detail previously (Goebels et al. 2015, Goebels et al. 2013). In the HKC, patients from the outpatient service with unilateral or bilateral keratoconus were included, as well as patients with possible corneal abnormality and patients without noticeable ocular abnormality but with thyroid diseases. Therefore, patients from the outpatient service of the Department of Endocrinology, Internal Medicine II, Saarland University Medical Center were also recruited. Exclusion criteria were previous ocular surgery or history of hydrops. All patients underwent a complete ophthalmological examination, including visual acuity test, refraction and slit lamp biomicroscopy. Medical history as well as history of contact lens (CL) wear was documented with respect to CL, the contact lens type (rigid or soft contact lens), as well as the time point starting with contact lens were noted.

Up to now, the database of HKC includes records of more than 800 patients. Incomplete records were excluded. If multiple records were available for one patient, we consequently considered the last examination.

Specular Microscopy

Measurements of the corneal endothelial cell density (ECD) were performed using the EM-3000 specular microscope (Tomey Corporation, Nagoya, Japan). With this noncontact photographic technique the corneal endothelium could be imaged with an optical magnification of 190 (Luft et al. 2015). Light is projected onto the cornea and the instrument captures the image which is reflected from the optical interface between the corneal endothelium and the aqueous humor. A sequence of 15 images is automatically captured during each measurement and up to 300 cells per image are counted within the region of interest by an automated image processing algorithm, implemented in the device (Luft et al. 2015). The image with the highest quality in terms of contrast and illumination is automatically selected by the instrument and subsequently verified manually by the examiner. We used the automated cell detection and counting implemented in the built-in manufacturer's software. Data collected from specular microscopy included cell size (minimum, maximum, average and standard deviation (SD), coefficient of variation (CV)), ECD, and corneal thickness (CT).

<u>Scheimpflug tomography</u>

Scheimpflug-based corneal tomography was performed using Pentacam® HR (Oculus Optikgeräte GmbH, Wetzlar, Germany). For classification of keratoconus stages we used the Topographic Keratoconus Classification (TKC) from Pentacam® HR, which is analogous to the Amsler-Krumeich classification.

In the Oculus Pentacam® a rotating camera captures the diffuse volume scattering of a monochromatic slit light source projected onto the cornea and the anterior eye segment. The software provides a series of keratoconus specific indices derived from topographic data. We selected the categorical TKC for keratoconus classification into grade 0 (normal) to grade 4 (severe). For intermediate stages provided by the Pentacam software (e.g. 2.5) we rounded the value up. In addition, we recorded metric parameters, such as the Keratoconus Index (KI) and Index of Surface Variance (ISV).

The data was collected between November 2010 and January 2015. Endothelial and topographic/tomographic measurements were performed by 5 trained nurses who are working as medical staff in our Department. The ophthalmological examinations, medical history, and informed consent of the patients to participate in the study were performed by certified ophthalmologists in our outpatient service.

Statistical analysis

Statistical analysis was performed using SPSS software (SPSS version 19.0, IBM, New York). Descriptive evaluation of the data was performed using mean, standard deviation (SD), median and minimum/maximum values. Correlations were tested using Pearson's rank correlation coefficient. P-values less than 0.05 were considered statistically significant. A nonparametric test was performed using the Mann-Whitney-U-Test.

Logistic regression analysis was used to investigate the effect of keratoconus (TKC stage) and contact lenses on the corneal endothelium.

Results:

The mean age of the 712 patients was 38 ± 15 (range 11 to 81) years. 66.7% eyes belonged to male patients; 48.7 % were left eyes. Mean uncorrected visual acuity (UCVA) was 0.39 ± 0.34, and mean best corrected visual acuity (BSCVA) was 0.72 ± 0.29.

According to TKC, 169 eyes (23.7%) were classified as normal, and 543 (76.3%) as keratoconus or keratoconus suspect. Out of these, 94 (13.2%) were classified as stage 1, 206 (28.9%) as stage 2, 166 (23.3%) as stage 3 and 77 (10.8 %) as stage 4. In **Table 1** the descriptive data are shown for ECD, coefficient of variation (CV), mean, SD, minimum and maximum cell area, corneal thickness, as well as the selected Pentacam® parameters KI, ISV and TKC (stages 0-4).

Data relating to CL wear were available in approximate 65% (461 out of 712) cases. Three groups of patients were specified wearing either no (n = 207), rigid (n=187) or soft (n=41) contact lenses. Thirteen patients reported wearing both soft and hard contact lenses – they were excluded from the subsequent calculations.
In 207 eyes the use of no contact lenses was documented (NoCL). Rigid contact lenses (RCL) were found in 200 eyes and were distributed as follows according to TKC-stage 0/1/2/3/4: 23/19/68/65/25. The wear of soft contact lenses (SCL) was found in 54 eyes, according to TKC-stage 0/1/2/3/4 the distribution was 17/9/19/6/3. In **Table 2** the descriptive data for ECD, CV, mean, standard deviation, minimum, and maximum of cell area, CT, as well as the selected Pentacam® parameters KI, ISV and TKC are shown for the three CL groups separately.

Evaluation of all eyes
Endothelial cell size, SD and coefficient of variation increased with increasing keratoconus stage. Cell density and corneal thickness decrease with increasing keratoconus stage (**Table 1, Figure 1-2**).

A significant correlation was found between keratoconus stage and all endothelial parameters. The keratoconus related indices TKC, ISV and KI correlated with minimum, maximum and mean cell size, standard deviation of cell size, cell density, coefficient of variation and corneal thickness (all $p<0.01$).

Evaluation of eyes regarding contact lens type

The correlations between the endothelial parameters and keratoconus indices separated into the three groups regarding contact lens (wearing RGP or soft contact lenses, or patients without contact lenses) wear are presented in **Table 3**. In patients without contact lenses a significant correlation was found between all endothelial parameters and the tested keratoconus indices ($p < 0.05$). Also here endothelial cell size, SD and CV increase with keratoconus stage, ECD and CT decrease with increasing keratoconus stage.

In patients with rigid contact lenses, CV does not change significantly with increasing severity of keratoconus ($p = 0.094$). Also no significant correlation was found between TKC/KI and maximum cell size ($p = 0.24$ / $p = 0.104$). ECD and CT do decrease with increasing keratoconus stage. In the SCL group only CT and minimum cell size show a significant correlation with keratoconus parameters of the pentacam ($p < 0.05$).

Comparing these three groups among each other the following results could be shown: Regarding endothelial cell density there was no significant difference between patients without contact lenses and patients wearing rigid or soft lenses. The coefficient of variation was significantly higher in patients with rigid contact lenses (45.1 ±17.2%) compared to patients without contact lenses (41.8 ± 12.4%) ($p = 0.022$), not between patients with soft contact lenses.

Discussion

Specular and confocal microscopic studies with small numbers of eyes show inconsistent results concerning the effect of keratoconus on corneal endothelial cell density (El-Agha et al. 2014, Hollingsworth et al. 2005, Ucakhan et al. 2006, Niederer el al. 2008, Mocan et al. 2008, Weed et al. 2007, Timucin et al. 2013). The literature results vary from lower to higher ECD in keratoconus. To our knowledge, this is the first study which investigates the effect of keratoconus severity on the corneal endothelium in a large study population of 712 eyes.

In the present study we found a significant decrease of ECD with progression of keratoconus. In addition we found a significant increase of CV of endothelial cell size with progression of the disease. The sizes of the smallest and largest cells both increased significantly. These results are comparable to the results from Ucakhan, Niederer and Mocan (Ucakhan et al. 2006, Niederer et al. 2008, Mocan et al. 2008).

The most recent study from El-Agha et al. reporting specular microscopy data of 40 keratoconic eyes showed a tendency to lower ECD and higher CV with advanced stages of keratoconus, but without statistically significant correlation (El-Agha et al. 2014). Using specular microscopy, Matsuda found an increase in the extent of polymegathism and increase of various cell shapes (Goebels et al. 2013) and pleomorphism in a study population of 21 keratoconic eyes.

Confocal microscopy in keratoconus shows controversial findings for endothelial changes (El-Agha et al. 2014). In one of the earlier studies, Hollingsworth et al. investigated 29 keratoconic corneas using confocal microscopy and found a 6% increase of ECD compared to normal control corneas (Hollingsworth et al. 2005). In contrast, two studies published in 2008 by Niederer et al. with a sample size with 52/52 eyes and Mocan et al. with 68/22 eyes found even lower ECD in keratoconic eyes compared to normals (Niederer et al. 2008, Mocan et al. 2008). Three studies report an unaffected ECD: Ucakhan et al. found a lower ECD without statistical significance in a series of 48 keratoconic and 44 normal eyes (Ucakhan et al. 2006). Weed et al. compared 19 keratoconic eyes with 38 normal eyes including 15 contact lens wearers and found normal ECD in moderate and severe keratoconus (Weed et al. 2007), and Timucin et al. described normal ECD in 65 keratoconus patients without contact lenses compared to 40 controls (Timucin et al. 2013). From these, the studies of El Agha et al., Niederer et al. and Timucin et al. explicitly included keratoconus patients without contact lens affection (El-Agha et al. 2014, Niederer et al. 2008, Timucin et al. 2013).

Four of these studies correlated ECD with staging of keratoconus. El-Agha et al. concluded that up to stage 3 keratoconus does not significantly affect the endothelium. Niederer et al. and Timucin et al. found no significant differences between mild to moderate and severe keratoconus (Niederer et al. 2008, Timucin et al. 2013). Only Ucakhan et al. found lower ECD in severe keratoconus (Ucakhan et al. 2006).

A decrease of ECD is amongst others reported to be related to CL wear (Bruce & Brennan 1990, Liesegang 2002). Stromal hypoxia, hypercapnia and thinning are well known to be associated with CL with low oxygen permeability, which should explain the relationship between CL and changes in corneal endothelium (Timucin et al. 2013, Liesegang 2002). Additional factors such as contact lens induced mechanical trauma may contribute to endothelial alteration (McMonnies 2014).

Our data show that in patients with keratoconus CV is significantly higher with RCL, whereas ECD does not differ significantly. With SCL the ECD is higher compared to eyes either with or without RCL, but this difference is not significant. CV does not differ significantly between our group with SCL and both other groups.

Other studies have demonstrated endothelial changes in patients using contact lenses in the 1980’s (Lee et al. 2001): for example, Matsuda compared the data of 14 keratoconic eyes with rigid contact lenses with keratoconic eyes without CL, and examination of the endothelium of the contact lens wearers showed a significantly higher coefficient of variation accompanied with a significant decrease in ECD in CL wearers (Matsuda et al. 1989). In a recent study by Lee et al. a significant decrease in ECD and a significant increase in the CV between healthy eyes and eyes using soft contact lenses were found (Lee et al. 2001).

In this study we have shown in a large number of patients that all endothelial parameters significantly correlate with the keratoconus stage in patients in which the cornea was unaffected by a CL and in the entire group of eyes.

The present study shows solid data concerning keratoconus patients, which are not affected by contact lenses. Further studies are necessary with respect to detailed information about the behavior of contact lens use such as special lens type, wearing time duration per day and the starting point of contact lens wearing.

In conclusion, we found significant changes of the corneal endothelium in a large keratoconus population of 713 eyes using specular microscopy. As endothelial cell density and corneal thickness decreases, the coefficient of variation of cell area, the minimum and maximum size of cells increase with the progression of keratoconus. In patients with rigid contact lenses compared to eyes without contact lens wear there is a statistically significant difference in the coefficient of variation, but endothelial cell density decreases in both groups significantly with increasing severity of keratoconus.

Funding/Support: None

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Figures

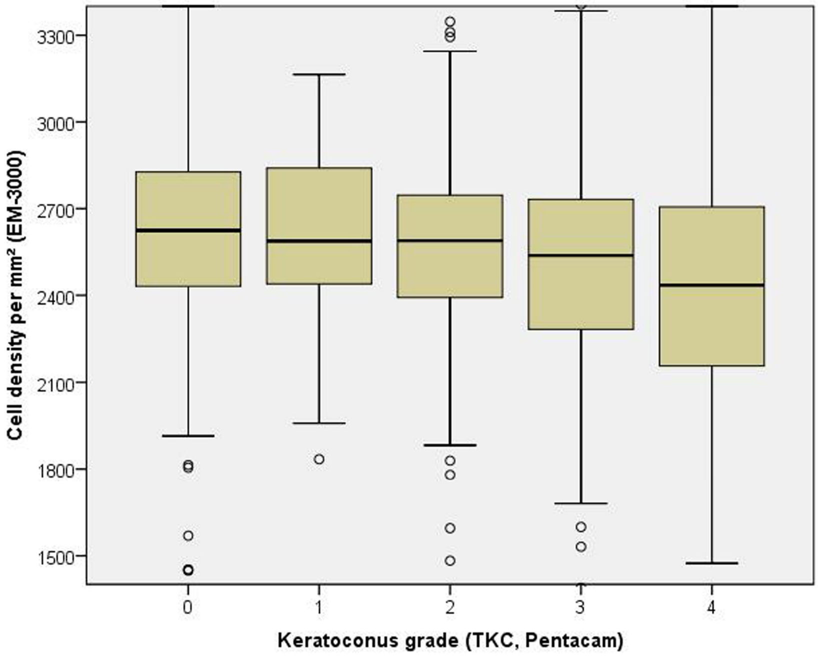

Figure 1: Endothelial cell density related to Topographic Keratoconus Classification (TKC, 0 healthy to 4 severe keratoconus). Cell density decreases with increasing severity of keratoconus (n=712).

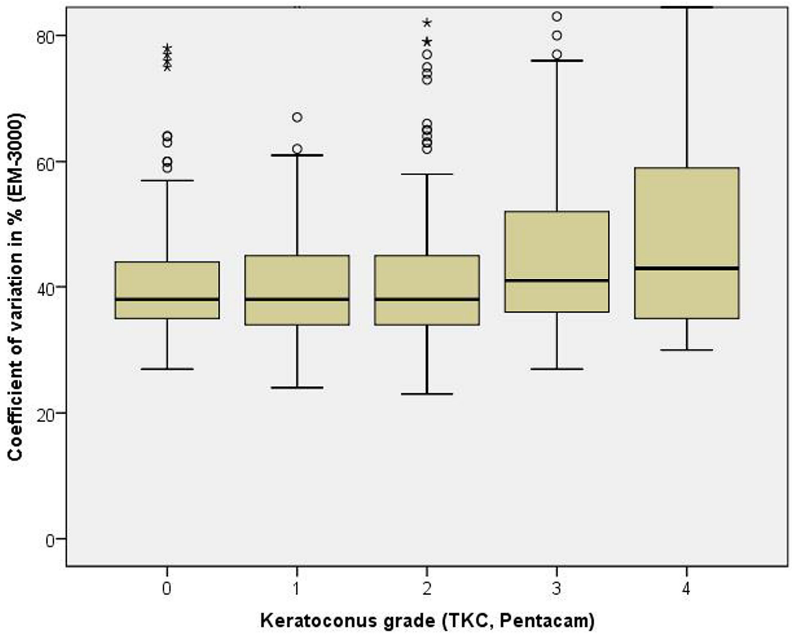

Figure 2: Coefficient of variation related to Topographic Keratoconus Classification (TKC, 0 healthy to 4 severe keratoconus). The coefficient of variation increases with increasing severity of keratoconus (n=712).

Table 1:

Mean, standard deviation, minimum and maximum of age, specular microscopy data and tomographic relevant data separated for the different keratoconus stages (0= healthy to 4=severe keratoconus)

		TKC 0 n = 169	TKC 1 n = 94	TKC 2 n = 206	TKC 3 n = 166	TKC 4 n = 77
	Age	42 ± 18 11-81	40 ± 14 11-76	35 ± 13 11-81	37 ± 13 11-78	34 ± 12 15-54
EM-3000	Minimum in µm² (cell size)	123 ± 25 73-223	126 ± 35 35-348	126 ± 25 71-215	128 ± 31 41-253	139 ± 39 85-336
	Maximum in µm² (cell size)	1029 ± 340 588-2836	1010 ± 971 614-2144	1081 ± 387 602-2953	1154 ± 534 552-4967	1200 ± 478 589-2701
	Mean in µm² (cell size)	391 ± 65 293-691	386 ± 47 316-545	399 ± 70 299-965	411 ± 80 248-879	437 ± 120 286-991
	SD in µm² (cell size)	164 ± 78 89-818	156 ± 50 88-426	170 ± 79 78-717	199 ± 124 98-1289	223 ± 125 105-614
	Cell density per mm²	2611 ± 356 1448-3413	2624 ± 300 1834-3165	2557 ± 327 1036-3347	2487 ± 379 1138-3406	2401 ± 464 1009-3503
	Coefficient of variation [%]	40.9 ± 11.2 27-119	40.0 ± 9.7 24-85	41.6 ± 11.8 23-94	46.2 ± 17.1 27-179	49.0 ± 17.0 30-111

	corneal thickness [µm]	**526.6 ± 35.3** **449-638**	**511.22 ± 44.06** **329-609**	**479.4 ± 43.4** **317 - 602**	**460.2 ± 45.0** **349-574**	**447.1± 46.3** **349-568**
Pentacam	**Central corneal thickness [µm]**	**544 ± 34** **471-686**	**523 ± 48** **266-613**	**495 ± 37** **375-583**	**471 ± 44** **225-591**	**448 ± 59** **241 - 512**
	Minimum thickness [µm]	**538 ± 35** **449-665**	**507 ± 60** **191-609**	**480 ± 36** **371 - 574**	**452 ± 46** **187-579**	**425 ± 56** **227-544**
	ISV	**18.4 ± 7.2** **7-44**	**42.2 ± 28.7** **17-226**	**65.76 ± 12.1** **46-134**	**104.94 ± 17.1** **33-190**	**152.45 ± 28.4** **54-269**
	KI	**1.0 ± 0.02** **0.9-1.1**	**1.1 ± 0.09** **0.95-1.7**	**1.2 ± 0.05** **1.0-1.37**	**1.3 ± 0.08** **0.9-1.5**	**1.4 ± 0.14** **1.0-2.1**

TKC: Topographic Keratoconus Classification

ISV: Index of Surface Variance

KI: Keratoconus Index

Table 2:

Mean, standard deviation, minimum and maximum of age, specular microscopy data and tomographic relevant data separated according to the wearing of no/rigid/soft contact lenses.

		No CL (NoCL) n=207	**Soft CL (SCL) n=41**	**Rigid CL (RCL) n=187**
EM-3000	Minimum in µm² (cell size)	128.9 ± 30.3 41-336	119.8 ± 31.1 35-209	128.9 ± 32.2 64-285
	Maximum in µm² (cell size)	1068 ± 384 552-2930	1035.0 ± 288.9 707-2237	1148.5 ± 517.5 602-4967
	Mean in µm² (cell size)	405.5 ± 70.6 248-762	385.9 ± 58.3 293-554	410.0 ± 101.1 294-991
	SD in µm² (cell size)	175.2 ± 88.6 88-717	144.7 ± 276.5 88-214	194.2 ± 131.7 78-1298
	Cell density per mm²	2523 ± 361 1313-3298	2644 ± 368 1805-3413	2533 ± 418 1009-3406
	Coefficient of variation [%]	41.8 ± 12.4 24-94	43.12 ± 9.8 31-77	45.1 ± 17.2 26-179
	corneal thickness [µm]	493.7 ± 44.8 382-608	498.7 ± 50.1 389-602	472.7 ± 49.9 333-638
Pentacam				
	ISV	64.4 ± 45.5 7-296	54.5 ± 35.6 13-145	84.9 ± 41.6 11-226
	TKC	1.5 ± 1.2 0-4	1.2 ± 1.0 0-4	2.1 ± 1.0 0-4
	KI	1.2 ± 0.2 1.0-1.7	1.1 ± 0.1 1.0-1.5	1.2 ± 0.1 0.9-1.7

CL: contact lens
TKC: Topographic Keratoconus Classification
ISV: Index of Surface Variance
KI: Keratoconus Index

Table 3:

Correlations between cell size, cell density, coefficient of variation, number of cells and central pachymetry and the keratoconus specific indices in all eyes separated into three groups. Those marked in bold are the parameters with significant correlations.

		NO CL (NoCL) (n=207)			**Soft CL (SCL) (n=41)**			**Rigid CL (RCL) (n=187)**		
		TKC	ISV	KI	TKC	ISV	KI	TKC	ISV	KI
Cell size [µm²]	Minimum	**p = 0.05**	**p = 0.001**	**p = 0.02**	p = 0.098	**p = 0.044**	**p = 0.007**	**p = 0.015**	**p = 0.001**	**p = 0.03**
	Maximum	**p = 0.01**	**p = 0.001**	**p = 0.04**	p = 0.842	p = 0.694	p = 0.773	p = 0.24	**p = 0.049**	p = 0.104
	Mean	**p ≤ 0.01**	**p ≤ 0.001**	**p ≤ 0.001**	p = 0.688	p = 0.598	p = 0,391	**p = 0.007**	**p < 0.001**	**p = 0.001**
	Standard deviation	**p ≤ 0.01**	**p ≤ 0.001**	**p ≤ 0.001**	p = 0.878	p = 0.619	p = 0,819	**p < 0.047**	**p = 0.05**	**p < 0.001**
Cell density per mm²		**p ≤ 0.01**	**p ≤ 0.001**	**p ≤ 0.001**	p = 0.496	p = 0.46	p = 0.378	**p = 0.004**	**p < 0.001**	**p < 0.001**
Coefficient of variation [%]		**p = 0.05**	**p ≤ 0.001**	**p = 0.001**	p = 0.760	p = 0.336	p = 0.851	p = 0.094	**p = 0.022**	p = 0.038
Central pachymetry [µm]		**p = 0.01**	**p ≤ 0.001**	**p ≤ 0.001**	**p = 0.11**	**p = 0.012**	**p = 0.003**	**p ≤ 0.001**	**p < 0.001**	**p < 0.001**

CL: Contact lens
TKC: Topographic Keratoconus Classification
ISV: Index of Surface Variance
KI: Keratoconus Index