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Role of corneal epithelial measurements in differentiating eyes with stable keratoconus from eyes that are progressing.

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Running head: Role of corneal epithelial measurements in keratoconus progression

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Key words: epithelium; keratoconus, progression
**Purpose:** To evaluate measures of corneal epithelium in eyes that showed documented signs of keratoconus (KC) progression and compare with stable eyes and healthy controls. Also, to determine the correlation of these epithelial parameters with keratometry ($K_{max}$) and pachymetry.

**Design:** Prospective observational comparative study.

**Participants:** One hundred and fifty eyes from 150 patients. The study included 50 eyes from patients with documented KC progression, 50 eyes with stable KC and 50 clinically normal eyes to serve as controls. The progressive KC group included eyes with an increase in maximum keratometry ($K_{max}$) of 1.00 diopter within 1 year.

**Methods:** A spectral-domain (SD) OCT imaging were obtained in all eyes, mean values were compared between the groups. Correlation of epithelial parameters with $K_{max}$ and thinnest pachymetry was also investigated.

**Main Outcome Measures:** For the purposes of this study, the epithelial measures maximum, minimum, superior, and inferior values as well as difference between minimum and maximum (min-max) and epithelial standard deviation were considered, obtained from SD-OCT and compared between groups. Measurements of thinnest point and min-max in pachymetry were also recorded.

**Results:** The only epithelial parameter that presented a statistically significantly difference between stable and progressive KC was epithelium min-max. While Stable KC presented epithelium min-max mean values of $-18.2 \pm 6.6$, progressive KC eyes presented mean values of $-23.4 \pm 10.3$ ($p<0.0001$). Epithelial maximum ($p=0.16$), minimum ($p=0.25$), superior ($p=0.28$), inferior ($p=0.23$), and standard deviation ($p=0.25$) values were not significantly different between stable and progressive eyes. Difference min-max pachymetry points in stable ($-108.3 \pm 33.5$) and progressive KC ($-115.2 \pm 56.0$) were not significantly different ($p=0.723$). There was no significant
correlation between epithelium min-max with corneal thinning (p=0.39) or k max(P=0.09) regardless of disease progression. Epithelium minimum and inferior presented a significant correlation with corneal thinning(p=0.0010 and p=0.0014 respectively) and kmax(p=0.0041 and p=0.0008 respectively) in eyes that are progressing.

**Conclusions**: Epithelial measures are useful to identify KC eyes that are progressing; Parameter that measures difference between minimum and maximum epithelium point were significantly different between stable and progressive groups, unlike this difference in pachymetry. Some cut-offs can potentially differentiate eyes with progression; Finally, this epithelial parameter seems to be independent of corneal thinning and k max.
Introduction:

Keratoconus (KC) is a structural and progressive disease, leading to changes in curvature and thickness and poor visual acuity.¹ A collective international effort group has been engaged in developing and validating new tools for diagnosing this disease at an increasingly early stage.²,³ With the advent and popularization of measures that interrupt its progression, avoiding or at least postponing a corneal transplant, it becomes essential to understand better these new parameters in eyes that are progressing, making viable intervention before the disease has more clinical severe repercussions.⁴

Studies show that there are marked epithelial changes in eyes with KC, and therefore the analysis of this layer can be helpful in the early diagnosis of this disease.⁵-¹⁰ From modifications in protein expression to structural alterations that, ultimately, represent thinning in steeper areas and adjacent thickening, it has recently been shown that the incorporation of epithelial analysis can increase the sensitivity for early diagnosis.¹¹,¹² An epithelial apoptosis process may explain the thinning of this corneal layer.¹¹

Recently, relevant factors on the KC natural history of the disease have been identified in a more methodologically appropriate design.¹ A steepening in the maximum keratometry (K max) above one diopter over one year is higher than expected for the natural history, being an essential sign of active progression.¹ While little or so is known about the behavior of the epithelial layer in KC that is actively progressing.

With wider mapping through spectral-domain optical coherence tomography (SD-OCT), it is currently possible to reproducibly measure parameters of the epithelial thickness.¹³,¹⁴ This study aims to identify measures of corneal epithelium that behave differently in stable keratoconus from those in progression.
Methods

Study Design and Subjects

This is a prospective comparative observational study approved by the institution’s ethics committee (University of Sao Paulo) and the Brazilian National ethics and research committee. This study also followed the tenets of the Declaration of Helsinki. Informed consent was obtained from all patients.

The study included patients with documented KC progression, patients with stable KC, and clinically normal eyes to serve as controls evaluated at private practice from January 2018 through January 2020. Inclusion criteria for cases comprised all patients with a documented diagnosis of KC. We excluded patients who presented allergic processes that were active. It is known that acute ocular allergic processes are related to the increase of inflammatory cytokines in tears, so acute allergy could be a confounding factor for epithelial measurements since the levels of inflammatory cytokines could be even higher depending on the severity of the allergy. The most prominent signs and symptoms were:

Eyelids: severe eczema of the eyelids and periorbital skin, prominent, periorbital darkening (allergic ‘shiners’), or the absence of the lateral eyebrow.

Conjunctiva: Papillary hypertrophy of the upper and lower tarsal conjunctiva. Gelatinous hypertrophy of the limbal conjunctiva (Limbal Horner-Trantas dots).

Cornea: Punctate epithelial keratitis, persistent epithelial defects, pannus formation, neovascularization, subepithelial scarring, lipid keratopathy.

Symptoms: Pain, redness, itching, burning of the eyes, foreign body sensation, and watery to thick ropy mucous discharge.
Patients with any other ocular pathologies, systemic inflammatory or autoimmune diseases, diabetes mellitus, with history of medications that increase hormone levels, pregnancy or with recent (less than 6 months) systemic/ocular allergy or infection history were excluded from the study. Patients using contact lenses were instructed to discontinue their use for a minimum of 4 weeks before the eye examination.

Patients using any type of anti-inflammatory/antiallergic ocular or systemic medications or who had undergone any ocular surgical intervention for both eyes were also excluded. Patients that had undergone a surgical intervention in only one eye were included in the study and the eye without surgery was included. All subjects also underwent a dry eye evaluation using Schirmer’s test, corneal staining with green lissamin and tear film break up time. Subjects with concurrent symptoms of dry eye were excluded.

**Patient selection**

The progressive KC group included eyes with an increase in K max of 1.00 diopter (D) within one year.\(^1\) The corneal topographic imaging was obtained using a Dual Scheimpflug device (Galilei G6, Ziemer Ophthalmic Systems AG) and were those the same indicated for corneal cross-linking.

The stable KC group consisted of patients with KC that did not meet objective criteria for progression.

Besides, a group of clinically normal eyes, paired by age, was also included to control. Although we used only one eye, clinically normal eyes had both eyes within normal standards. In this group, the presence of subclinical ectatic corneal disease was excluded bilaterally. Data collected included gender, age, the patient’s ocular, and medical history. Again, careful attention was paid to evaluate and exclude the presence of a clinical history of atopy. In addition, all patients
were requested to answer a questionnaire on eye rubbing, irritation, and pain in their eyes to rule out ocular allergy.

For all groups, only one eye per patient was included in the study. When the patient had one eye in progress, and the other eye stable, only the eye in progress was considered for this study.

All patients underwent a complete ophthalmological examination, including uncorrected distance visual acuity (UCVA), best spectacle-corrected visual acuity (BSCVA), and manifest refraction. The same examiner (LRS) carefully performed the refraction. UCVA and BSCVA were measured in decimal Snellen and converted to the logarithm of the minimum angle of resolution (logMAR) for statistical analysis.

The stage of the disease was defined by the Amsler-Krumeich classification using the parameters of corneal curvature (average curvature in the central 3 mm of the cornea), refractive error (degree of myopia/astigmatism), pachymetry (central corneal thickness), and biomicroscopy (transparent cornea or with opacities and/or perforation). This classification is not associated with any specific device and allows comparison with previous studies.

Corneal curvature and power, thickness maps, and elevation parameters were evaluated using the same Dual Scheimpflug analyzer device previously described and according to the manufacturer’s guidelines. Only measurements that satisfied the minimum quality required by the system were included. The same experienced examiner acquired all the images (MRS).

A SD-OCT system (Avanti; OptoVue, Inc.) with a corneal adaptor lens was used to acquire pachymetry (corneal thickness) and epithelial thickness maps. It has a working wavelength of 840 nm and operates at a scan speed of 70 000 axial scans per second. Equipped with an add-on lens, this system makes corneal measurements with the "PachymetryWide" scan mode, consisting of B-scans evenly in eight radial directions at a length of 9 mm centered at the pupil.
center. The device was used according to the user's manual. The scans were triggered manually after the alignment procedure was completed. Participants were asked to sit back to ensure the measurement independence, and the scan unit was thoroughly reset before each subsequent scan. The data were valid if the measurement outcomes showed sufficient image signals and good quality. According to the device display, the epithelial data was obtained within the 7 mm zone.

Epithelial measurement (μm) parameters obtained from SD-OCT and compared between groups for this study:

1. Epithelial Min: Minimum epithelial thickness of the map
2. Epithelial Max: Maximum epithelial thickness of the map
3. Epithelial Min-Max: Difference between minimum and maximum epithelial thickness of the map
4. Epithelial Std Dev: Standard deviation of the epithelial thickness of the map
5. Epithelial Superior: Average epithelial thickness of the superior region of the map
6. Epithelial Inferior: Average epithelial thickness of the inferior region of the map

Measurements of thinnest point and difference between minimum and maximum in pachymetry points were also recorded from OCT for comparative purposes. Three consecutive measurements were performed by an experienced operator (MRS). Correlation of epithelial parameters with K max and thinnest pachymetry was also investigated.

Statistical Analysis

The results obtained were expressed as mean ± standard deviation (SD). Variance analyses (ANOVA) were used to compare mean results in the same group and Tukey multiple pairwise comparisons were performed for comparison between groups. (Tukey Honest Significant
Differences, R function: Tukey HSD). All statistical analysis were performed with JMP 16 statistical software. The mean values, standard deviation (SD) and 95% confidence intervals (CIs) were determined. To correct for multiple comparisons performed in this study (approximately 18) using the Bonferroni method, only individual p values less than .003 were considered significant. Graphic expressions were elaborated by box plots and density distribution of values. Sample size calculation was performed to detect differences of at least 2 μm in epithelial thickness measurements between stable and progressive keratoconus, at a significance level of 5% and a power of 80%, assuming a standard deviation of 10%. The minimum sample size of this study was 50 eyes per group.

While Epithelial Min-Max is a continuous variable, an analysis of the receiver operating characteristic (ROC) curve was also made to determine potential cut-off values with their respective sensitivity and specificity. Pearson correlation (r) was used to measure linear dependence between two different variables (x and y). The plot of y = f(x) is represented by the linear regression curve. The direction and strength of Pearson correlation were interpreted as follows: 0.9 to 1.00 (-0.90 to -1.00) very highly positive (negative) correlation; 0.70 to 0.89 (-0.70 to -0.90) highly positive (negative) correlation; 0.40 to 0.69 (-0.40 to -0.69) moderately positive (negative) correlation; 0.20 to 0.39 (-0.20 to -0.39) low positive (negative) correlation; 0.00 to 0.19 (0.00 to -0.19) negligible correlation. A p value < 0.05 was considered statistically significant for the correlation.

Categorical variables were compared using either chi-square or Fisher exact test as appropriate. Categorical variables were expressed as percent (%).

**Results:**
This study included 150 eyes of 150 patients, being 50 with progressive KC, 50 stable and 50 clinically normal eyes (controls). There were no significant differences between groups for age (22.7 ± 4.09 years in the progressive KC group vs. 23.3 ± 4.31 years in the stable KC group vs. 23.6 ± 6.13 years in the control group; p=0.811) or sex distribution (51% male patients in the progressive KC group: 52% male patients in the stable KC group, 54% male patients in the control group (p = 0.762).

The groups were comparable in terms of disease stage: 58% (29 eyes) stable group vs. 50% (25 eyes) in the progressive group were classified as stage I (p 0.654); 22 % (11 eyes) stable group vs. 24% (12 eyes) in the progressive group were classified as stage II (p 0.813); 4% (2 eyes) stable group vs. 4% (2 eyes) in the progressive group were classified as stage III (p 1.0), and 16% (8 eyes) stable group vs. 22% (11 eyes) in the progressive group were classified as stage IV (p 0.446).

Table 1 shows mean values and standard deviation of epithelial measurements in all groups. The only parameter that presented a statistically significantly difference between stable and progressive KC was epithelium Min-Max. While stable KC presented epithelium Min-Max mean values of -18.2 ± 6.6μm, progressive KC eyes presented mean values of -23.4 ± 10.37μm (p<0.0001). Figure 1A.

The ROC curve analysis to separate eyes with stable keratoconus and eyes that are progressing through the epithelial min-max variable revealed some cut-off values and their respective sensitivity and specificity. For example, for a value of 23.4μm, the sensitivity was 84%, while the specificity was 46%. The value of 31μm min-max difference reached a sensitivity of 98%. Figure 2.
Measurements of difference between minimum and maximum in pachymetry points in stable (-108.3 ± 33.5µm) and progressive KC (-115.2 ± 56.0µm) were not significantly different (p = 0.723). Figure 1B.

Table 2 shows the correlation of epithelial parameters with K max. In stable KC eyes there is a significant correlation between measurements of epithelium Min. (pearson = -0.36, p = 0.0218) and epithelium Inferior (pearson = -0.40, p = 0.0088) with K max. Also, in progressive KC eyes there is the same significant and negative correlation between epithelium Min (pearson = -0.40, p = 0.0041) and epithelium Inferior (pearson = -0.46, p = 0.0008) measurements with K max.

Table 3 shows the correlation of epithelial parameters with thinnest pachymetry. While in stable eyes there is a significant correlation between measurements of epithelium Max. (pearson = -0.33, p = 0.0327), epithelium Min-Max (pearson = 0.33, p = 0.0331), epithelium Superior (pearson = -0.46, p = 0.0025) and epithelium Std Dev (pearson = -0.41, p = 0.0071) with thinnest pachymetry, in progressive eyes the significant correlation is only with epithelium Min. (pearson = 0.45, p = 0.0010) and epithelium Inferior (pearson = 0.44, p = 0.0014).

There was no significant correlation between epithelium Min-Max with corneal thinning (p=0.39) or K max(P=0.09) regardless of disease progression. Epithelium Min. and Inferior presented a significant correlation with corneal thinning (p=0.0010 and p=0.0014 respectively) and K max (p=0.0041 and p=0.0008 respectively) in eyes that are progressing.

Figure 3 is an illustrative picture revealing epithelial Min-Max values according to different stages in both groups, eyes with stable keratoconus and eyes that are progressing.
Discussion:

The main findings of this study are that epithelial measures are useful to identify eyes with actively progressing keratoconus; the difference between the minimum and maximum epithelial points were significantly different between stable and KC groups and some cut-offs can potentially differentiate eyes with progression, unlike this difference in pachymetry; and that this epithelial change seems to be independent of changes in the K max and the thinnest pachymetric point of the cornea. Figure 4.

Previous studies had already revealed the importance of epithelial parameters in the diagnosis of KC, probably relating to the fact that these alterations are associated with early microbiological disturbances. Therefore, we hypothesized that there could be noticeable differences in this layer in eyes that showed active disease progression compared to stable eyes, regardless of their stage.

A possible explanation for the role of the more expressive Min-Max epithelial difference in eyes with progression is that the corneal epithelium has rapid cell turnover and is highly reactive to asymmetries in the shape of the underlying stromal surface. Direct measurements of the remodeling of the epithelial layer can, therefore, suggest progression. This study also confirms that more meaningful than punctual values such as epithelial thinnest point, metrics associated with the asymmetric reactive capacity of the epithelium are capable of detecting subtle differences between groups.

The meaningful difference in the Min-Max variable between stable and progression eyes is even more interesting as there is no significant correlation between this epithelial parameter and corneal thinning or K max regardless of disease stage. In other words, this variable behaves independently and, therefore, can be a valuable tool in monitoring these patients. While ROC curve
analysis shows that the combination of sensitivity and specificity is limited for values below 23 microns, values above 23\(\mu\)m and, especially, above 31 \(\mu\)m are more sensitive in the possible detection of eyes that may be actively progressing.

Though not evaluating progression, previous studies found that epithelial thickness measurements from OCT have value in early diagnosis of forme fruste keratoconus. Hwang et al.\textsuperscript{10} showed that epithelial thickness variability metrics (epithelial Min-Max and epithelial Std Dev) were among the most valuable parameters distinguishing eyes with early stages of KC from normal populations. Li et at.\textsuperscript{17} found epithelial thickness Std Dev to be a strong predictor of early keratoconus, and Temstet et al.\textsuperscript{18} found epithelial thickness in the corneal thinnest location useful in the diagnosis of early subclinical keratoconus.

Another relevant finding of this study is that although there is a significant difference in the Min-Max measurement of the epithelial layer, the same does not occur in the Min-Max of pachymetry, emphasizing that pachymetric map (stromal + epithelial) measurement is not sensitive to structural changes that occur as KC progresses.

This finding explains that the pachymetric map does not necessarily correspond to the stromal thickness since the epithelium is distributed irregularly in the keratoconus.\textsuperscript{6,7,19} The thinnest epithelium point follows the thinnest stroma only when this area coincides with the steeper curvature, as one of the inducers of epithelial remodeling is the change in the shape gradient.\textsuperscript{6,7,19}

The finding of greater Min-Max variability in eyes with progression compared to stable eyes is even more relevant given the concomitant finding of the absence of correlation of this parameter with K max. This dissociation is explained by changes associated with the primary process of epithelial apoptosis in corneas with KC, regardless of curvature changes.\textsuperscript{11,12}
Wang et al.\textsuperscript{11} investigated the histopathology of epithelia and its micro-ribonucleic acid (miRNAs) regulation in eyes with KC. They resolved the histological structure of the keratoconic corneal epithelium and identified cell apoptosis, altered cell integrity, and down-regulation of miRNAs as potential mechanisms for keratoconic corneal epithelial degeneration. In addition, the apoptosis-related marker, p53 protein, was up-regulated in the keratoconic corneal epithelium, suggesting degeneration of this layer.\textsuperscript{11} Shetty et al.\textsuperscript{12} showed that the structural deformity of the KC cornea strongly correlates with reduced epithelial expressions of collagen fibril-maturing enzyme lysyl oxidase and that KC corneal epithelium expresses high levels of matrix metalloproteinase 9.

The process of apoptosis occurs asymmetrically in these corneas, so it makes sense that measures of epithelial variability play a role in differentiating early cases vs. normal corneas and distinguishing eyes in active progression vs. stable eyes, the main finding in our article.

Although it did not help differentiate between stable and progressive eyes, which is the objective of this study, we emphasize a correlation between the thinning of the epithelium, mainly in the cornea's inferior region, with the increase in the K max. This finding corroborates previous studies, which were more oriented toward the epithelium as a diagnostic tool.\textsuperscript{18,20}

Using an OCT apparatus whose epithelial measurements are derived from a smaller (5 mm) mapping diameter, Serrao et al.\textsuperscript{21} found the inferior paracentral region of the corneal epithelium to be significantly thinner in progressive than stable KC. Also utilizing a smaller diameter OCT mapping, Ouanezar et al.\textsuperscript{22} found no epithelial differences in progressing eyes compared to stable eyes. In addition to the smaller diameter that can influence the outcomes, it would have been critical that the authors had ruled out active allergic changes on the surface, as we did in our study, especially in stable eyes, since this could influence measures of epithelial variability. This way,
we have access to epithelial alterations with a lower risk of bias. These methodological differences explain the differences in findings. Our study went further by ruling out allergic bias and accessing a larger diameter OCT mapping. It detected the most significant variability between the thinning of one area and the thickening of another in progressing eyes. Due to the nature of the disease, maps with a larger image capture diameter used in our study are more sensitive to these measures of epithelial variability.

This study has some limitations. First, there is significant variability among individuals who are actively progressing both in objective measures and in the speed of this progression. However, most of the variables and variable categories identified as critical in our study will prove essential in other analyses, even if possibly by varying degrees, as we investigate measures (epithelial) that have been proven to change early.

Furthermore, the publication of these findings would represent a positive first step toward directing future studies in this context. Another possible limitation is that representative tensile strength measurements or biomechanics were not performed. Future studies that include these analyses in a reproducible manner may contribute to identifying patients with a greater propensity for progression.23

One of the aspects highlighted in this article is that we must separate the groups into stable vs. progression to investigate variables associated with progression adequately.24 Staging is a static assessment, whereas "being in progression" indicates a dynamic evaluation. For example, there are stage I eyes that are progressing and stage IV eyes that are stable. In the article's illustrative figure (Figure 4), we present a grade I keratoconus that is actively progressing. In other words, the stage does not represent a measure of active progression if used in isolation. Therefore, it does not
allow for adequate correlations or identifying variables associated with dynamic changes. The main indication of crosslinking is not a disease stage, its active progression.\textsuperscript{4}

Hence, determining a variable (difference between the min-max thickness of the corneal epithelium) associated with eyes that are progressing represents a step forward in our knowledge. Moreover, that can even, as illustrated the figure 4, be identified in eyes with early stages of the disease.

In conclusion, this study shows that although epithelial thinning measures are helpful in the diagnosis, it is a measure that reveals epithelial variability as the most useful in detecting eyes that are actively progressing compared to stable KC ones. Furthermore, some variability cut-offs can differentiate eyes with progression with relatively high sensitivity. The epithelium Min-Max measure can aid monitoring and eventually suggest the indication of corneal crosslinking before the significant visual loss.
References:


Figure legends:

**Figure 1:** Box-plot graph: A. Difference between minimum and maximum (Min-Max) epithelial thickness of the map (epithelial Min-Max) values comparing progressive keratoconus (KC), Stable KC and healthy controls. The medium of the epithelial Min-Max values of the progressive group is significantly higher than stable group and controls (p<0.0001). B. Measurements of difference between minimum and maximum in pachymetry (Pachymetry Min-Max) points in stable and progressive KC were not significantly different (p = 0.723). The bar inside each box represents the median and each box extends from the 25th percentile to the 75th percentile of the distribution in each group.

**Figure 2:** Receiver operating characteristic (ROC) curve: Analysis of the ROC curve to separate eyes with stable keratoconus and eyes that are progressing through the epithelial min-max variable revealed some cut-off values and their respective sensitivity and specificity. For example, for a value of 23.4μm, the sensitivity was 84%, while the specificity was 46%. The value of 31μm min-max difference reached a sensitivity of 98%.

**Figure 3:** Illustrative bar graph revealing difference between minimum and maximum epithelial thickness values (Epithelial Min-Max) according to different stages in both groups, eyes with stable keratoconus and eyes that are progressing.

**Figure 4:** Representative image of an eye with keratoconus progression of maximal keratometry documented in one year, with high variability of the epithelium min-max parameter (red arrow), although presenting epithelium minimum and standard deviation values similar to that of stable KC. Figure shows dual Scheimpflug imaging with total corneal thickness pachymetric map and anterior curvature (top row), and spectral-domain optical coherence tomography with total corneal thickness pachymetric map and epithelial thickness map (bottom row).
Table 1: Comparison of epithelial parameters between control, stable and progressive keratoconus

<table>
<thead>
<tr>
<th>Epithelial parameters (µm)</th>
<th>Patient group</th>
<th>Comparisons (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy Subjects</td>
<td>Stable Keratoconus</td>
</tr>
<tr>
<td>Epithelium Minimum</td>
<td>47.6 ± 4.9</td>
<td>42.7 ± 5.7</td>
</tr>
<tr>
<td>Epithelium Maximum</td>
<td>56.0 ± 3.5</td>
<td>60.9 ± 6.5</td>
</tr>
<tr>
<td>Epithelium Min - Max</td>
<td>-8.4 ± 3.6</td>
<td>-18.2 ± 6.6</td>
</tr>
<tr>
<td>Epithelium Superior</td>
<td>51.7 ± 3.6</td>
<td>53.0 ± 5.6</td>
</tr>
<tr>
<td>Epithelium Inferior</td>
<td>53.1 ± 4.1</td>
<td>53.7 ± 3.8</td>
</tr>
<tr>
<td>Epithelium Standard deviation</td>
<td>1.6 ± 0.4</td>
<td>4.5 ± 1.8</td>
</tr>
</tbody>
</table>

* statistically significant difference
| Epithelial parameters | Stable Keratoconus | | | | Progressive Keratoconus | | | |
|-----------------------|--------------------|--------|--------|--------------------------|--------|--------|--------|
|                       | Correlation | Lower 95% | Upper 95% | P value | Correlation | Lower 95% | Upper 95% | P value |
| Epithelium Minimum    | -0.36        | -0.60    | -0.05    | 0.0218* | -0.40        | -0.61    | -0.14    | 0.0041* |
| Epithelium Maximum    | -0.20        | -0.49    | 0.11     | 0.19     | -0.05        | -0.32    | 0.23     | 0.73 |
| Epithelium Min - Max  | -0.10        | -0.40    | 0.21     | 0.51     | -0.24        | -0.48    | 0.04     | 0.09 |
| Epithelium Superior   | -0.12        | -0.42    | 0.19     | 0.45     | -0.03        | -0.31    | 0.25     | 0.81 |
| Epithelium Inferior   | -0.40        | -0.64    | -0.11    | 0.0088* | -0.46        | -0.65    | -0.20    | 0.0008* |
| Epithelium Standard deviation | 0.18 | -0.13    | 0.46     | 0.27     | 0.15         | -0.13    | 0.41     | 0.29 |

* statistically significant difference
### Table 3: Correlation of epithelial parameters with thinnest pachymetry

| Epithelial parameters | Stable Keratoconus | | | | | | | | Progressive Keratoconus | | | | | |
|-----------------------|--------------------|---|---|---|---|---|---|---|--------------------|---|---|---|---|
|                       | Correlation        | Lower 95% | Upper 95% | P value | Correlation | Lower 95% | Upper 95% | P value |                 | Lower 95% | Upper 95% | P value |                 |
| Epithelium Minimum    | 0.00               | -0.30     | 0.31      | 0.96     | 0.45        | -0.61     | -0.14     | 0.0010*  |
| Epithelium Maximum    | -0.33              | -0.59     | -0.03     | 0.0327*  | 0.25        | -0.02     | 0.50      | 0.07     |
| Epithelium Min - Max  | 0.33               | 0.03      | 0.59      | 0.0331*  | 0.12        | -0.15     | 0.38      | 0.39     |
| Epithelium Superior   | -0.46              | -0.68     | -0.18     | 0.0025   | 0.21        | -0.07     | 0.47      | 0.13     |
| Epithelium Inferior   | 0.11               | -0.20     | 0.40      | 0.49     | 0.44        | 0.18      | 0.64      | 0.0014*  |
| Epithelium Standard deviation | -0.41          | -0.64     | -0.12     | 0.0071*  | -0.07      | -0.34     | 0.21      | 0.61     |

* statistically significant difference
Epithelial Min-Max value cutoff

31.1 μm sensitivity 98% specificity 32%
23.4 μm sensitivity 84% specificity 46%
18.2 μm sensitivity 60% specificity 54%
Epithelial measures are useful to identify keratoconus eyes that are progressing; The difference between minimum and maximum epithelium point was significantly different between stable and progressive groups. Some cut-offs can potentially differentiate eyes with progression.