Original article

Corneal morphometric predictive models from ametropia to excimer laser treatment

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\textbf{A B S T R A C T}

Objective: To develop corneal morphometric models with refractive error in excimer laser surgery.

Method: A prospective-longitudinal study was conducted on 78 patients (151 eyes) using the LASIK surgical technique, and 56 patients (111 eyes) with myopic astigmatism using ESIRIS (Schwind-Germany) equipment with pendulous microkeratome. The results were analyzed using descriptive statistics. A NIDEK Confoscan microscope was used to obtain and study the images.

Results: After LASIK treatment 84.3\% of the variations in epithelium thickness variations were due to the magnitude of refractive error and the epithelium thickness before LASIK treatment. More than two-thirds (68.8\%) of the variations in keratocyte density variations in posterior flap and 48.2\% of the variations in the anterior retroablation zone were due to the magnitude of the refractive error. Variations of 90\% were found in the corneal thickness after LASEK, which were due to the magnitude of the refractive error before LASEK.

Conclusions: Predictive models reveal that morphometrical variations depend on the magnitude of the refractive error. These models are very important in the selection of patient for refractive surgery, and also for the specific technique to use.

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Modelos predictivos de morfometría corneal a partir de la ametropía a tratar con láser excimer

**Resumen**

Objetivo: Desarrollar modelos predictivos de morfometría corneal en cirugía refractiva con láser excimer para la corrección de ametropías.

Método: Se realizó una investigación longitudinal y prospectiva con 78 pacientes (151 ojos) operados con LASIK y 56 pacientes (111 ojos) operados con LASEK. Se utilizó el microscopio confocal ConfoScan 4 de NIDEK. Se aplicó el ANOVA de un factor con corrección de Bonferroni, correlación de Pearson y análisis de regresión lineal múltiple con validación cruzada.

Resultados: Tras el LASIK, el 84,3% de las variaciones del grosor epitelial se deben a la magnitud de la ametropía tratada y al grosor epitelial preoperatorio. El 68,8 y el 48,2% de las variaciones de la densidad de queratocitos en el colgado posterior y zona de retroabación posterior, respectivamente, se deben a los valores de estas variables antes de LASIK y a la magnitud de la ametropía tratada. Tras el LASEK el 90 y el 53% de las variaciones de paquimetría corneal y densidad de queratocitos al año, respectivamente, se deben al valor de esta variable en el preoperatorio y a la magnitud de la ametropía tratada.

Conclusión: Los modelos predictivos obtenidos revelan que las variaciones de las variables morfométricas al año del tratamiento dependen en gran medida de sus valores preoperatorios y de la magnitud de la ametropía a tratar. Estos modelos constituyen herramientas a tener en cuenta como criterios de selección de pacientes candidatos a cirugía con láser excimer para tratamiento de ametropías, y para la elección óptima de la técnica quirúrgica.

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**Introducción**

Refraction disorders (myopia, farsightedness, astigmatism) are among the most frequent vision alterations, with prevalence rates varying with age, country, ethnic group, education level and occupation. Some articles report that 30% of the population in Western countries exhibit myopia. However, higher percentages are reported in Asian countries, reaching up to 50%. In the United States, 25% of the population between 15 and 24 exhibit some refractive defect. In Segovia, the prevalence of myopia reaches 23% and in other cities of northern Europe it rises to 40%. Several studies suggest the possibility that this defect may increase in the next few years.

A study carried out in Cuba revealed a higher frequency of compound myopic astigmatism among ametropia. In Pinar del Río, 23% of the population exhibits a refractive defect according to data obtained during MisióMilagro.

The significant scientific and technological developments in ophthalmology, particularly in refractive surgery, and the existence of new high impact laser devices together with progress made in optic microscopes, have enabled a new dimension of morphophysiological concepts on corneal tissues facilitating the study of conditions which a few years ago were unknown.

The success of said procedures is undeniable in what concerns visual results, quick recovery and diminished possibilities of trans- and post-surgery complications. However, when the curvature of the cornea is modified, a new architecture is created which, even though it may achieve high vision standards for patients, tissue response must be taken into account as well as the morphometric modifications occurring in cornea that have received such treatment. This is of great importance as it represents a fundamental variable for evaluating the safety, effectiveness and predictability of this technology, even more so taking into account that in the development of the scientific and technological breakthroughs leading to said surgical techniques, some have been set aside due to their collateral results.

At the international level, several researchers have published the morphometric changes occurring after treatment with Excimer laser, but no predictive models of variables have been developed. These would enable ophthalmologists to determine beforehand the corneal morphometry in order to guarantee the evaluation and safety of ametropia correction with Excimer laser. The present research aims at developing predictive models of morphometric variables based on the magnitude of the ametropia to be treated.

**Temas, material and methods**

An observational, descriptive, longitudinal and prospective research was carried out in the Ophthalmology Department of the Abel Santamaría Cuadrado Hospital in Pinar del Río. The study comprised patients who visited the refractive surgery practice and fulfilled the study criteria. The research comprises 2 universes:

- Universe 1: Patients intervened with the LASIK surgery technique.
• Universe 2: Patients intervened with the LASEK surgery technique.

Both universes fulfilled the following criteria:

Inclusion criteria

Age over 20, with 2-year refractive stability. Refractive defects: myopia up to 8 diopters, astigmatism up to 4 diopters, compound myopic astigmatism under 8 diopters (in algebraic addition of sphere and cylinder), uncorrected visual acuity of 0.5 or less. Visual acuity with correction in the eye with less vision above 0.3, programmed residual corneal bed above 300 μ in LASIK and above 400 μ in LASEK, mean initial and final programmed keratometry between 36 and 48 diopters, mean initial and final programmed keratometry between 36 and 48 diopters. Presurgery pachymetry above 500 μ, normal corneal topography.

Exclusion criteria

General
Uncooperative patients in confocal microscopy, patients who did not attend some of the scheduled visits of the study or did not give their consent for participating therein, systemic diseases such as diabetes mellitus, epilepsy, collagen diseases, immunodepression, psychiatric disorders, Marfan syndrome, Ehlers Danlos syndrome, psoriasis and allergies, systemic diseases, pregnancy and puerperium (up to 6 months).

Ocular exclusion criteria

Single eye; alteration of ocular or tear annexes (infection, inflammation, dry eye); abnormal arbitrary configurations (small or deep orbits, small palpebral fissure, enophthal-mos, prominent brow); previous corneal disease (keratitis due to herpes simplex, zoster, confirmed or suspected corneal ectasia, relapsing corneal erosions, leukemia, pannus, dystro phy, degenerations); use of contact lens in the year prior to the study, strabismus or previous operation thereof, previous refractive corneal surgery, glaucoma or ocular hypertension, lens sclerosis or cataract, uveitis, retinal diseases (tears, retina detachment history, vitrectomy, macular degeneration, pigmented retinosis).

The sample was constituted by all the patients (134) who attended the refractive surgery practice and fulfilled the inclusion criteria from November 1, 2010 to May 31, 2011; overall, 78 patients (151 eyes) operated with LASIK and 56 patients (111 eyes) operated with LASEK.

Images constituting reference limits were previously defined by means of confocal microscopy in order to render the variables operative (Fig. 1).

The following variables were analyzed:

1. Pachymetry (continuous quantitative): measured with full focus confocal microscopy, represented as depth in the left superior part of the CMTF curve, expressed in μ.
2. Epithelial thickness (continuous quantitative): measured with full focus confocal microscopy from the first apical corneal epithelium image up to the last visible base corneal epithelium image, expressed in μ.
3. Flap thickness (continuous quantitative): measured in patients operated with LASIK, by means of full focus confocal microscopy, from the first image of the apical corneal epithelium up to the first surgical interface image, expressed in μ. The value of the presurgery flap thickness corresponds to the value programmed in all cases, i.e., 160 μ.
4. Residual stromal bed (continuous quantitative): measured in patients operated with LASIK, by means of full focus confocal microscopy from the last surgical interface image up to the last corneal endothelium image, expressed in μ. The presurgery residual corneal bed value corresponds to the value shown by the ORK-CAM application for aspherical treatments utilized for programming cases, resulting from the subtraction of 160 μ (programmed flap thickness) to the presurgery pachymetry value measured with confocal microscopy.
5. Apical epithelium cell density (quantitative continuous): determined with a manual method, expressed in cells/mm². The apical corneal epithelium image described above was selected. Each cell was marked in a rectangular area (A) of 0.0500 mm² to prevent double counting, intercepted with the limits of the area, counted only in the superior and nasal sector. N was defined as the number of cells counted in one area (A). Density was expressed in cells per mm², obtaining the quotient between N and A.
6. Basal epithelium cell density (quantitative continuous): determined with a manual method, expressed in cells/mm². The basal corneal epithelium image described above was selected. Each cell was marked in a rectangular area (A) of 0.0500 mm² to prevent double count. The cells intercepted by the area limits were counted only in the superior and nasal sector. N was defined as the number of cells counted in one area (A). Density was expressed in cells per mm², obtaining the quotient between N and A.
7. Keratocyte density (quantitative continuous): amount of keratocytes per mm³. A keratocyte was defined as a shiny and elongated object (keratocyte nuclei) appearing in corneal stroma images over a black background. Keratocyte density for each stromal sub-layer was calculated. In a predefined rectangular area (A) of 0.0500 mm² each elongated shiny object was marked (assumed as keratocytes nuclei). In order to prevent double count, shiny objects intercepted by the limits of the area were counted only in the nasal and superior portion. N was defined as the number of objects counted in one area (A). Density was expressed in cells per mm³, obtaining through software a first quotient between N and A. Subsequently, said value was divided by the effective field depth of the utilized microscope: 25. Keratocyte density was obtained in different corneal stromal regions. Several stromal sub-layers were defined in accordance with their level of depth, the surgical technique applied and the time of measurement (either pre-or post-surgery). Each stromal sublayer is represented by a selected image. In patients operated with LASIK the following stromal sub-layers were selected (Fig. 2):
- Anterior flap: defined as the most anterior sub-layer of the corneal stroma in presurgery and the anterior half of the stromal flap in post-surgery. Represented by the
Fig. 1 – Corneal sub-layers seen with confocal microscopy. (A) Apical corneal epithelium: polygonal cells layer with well defined edges, shining nuclei which stands out over the homogeneous cytoplasm. Image taken of a patient 6 months after LASEK. (B) Baseline corneal epithelium: layer of cells with darker homogeneous cytoplasm lacking nuclei and defined edges. Image taken of a patient one year after LASEK. (C) Sub-basal nerve plexus: nerve fibers which stand out from the dark background, thin, shiny, in parallel or oblique distribution with several interconnecting bifurcations. Image of a patient taken one year after LASIK. (D) Corneal stroma: defined by the presence of shiny oval objects (keratocytes) standing out from the dark background. Image of a patient taken 3 months after LASIK. (E) Surgical interface: presence of dot-like shiny objects standing out from the dark background. Image of a patient taken one month after LASIK. (F) Corneal endothelium image: hexagonal cells with defined edges, without nuclei and homogeneous cytoplasm. Image of a patient taking one year after LASEK. (G) Corneal haze image, keratocyte limits cannot be defined, more shiny than the rest of stromal images. Image of a patient taken 3 months after LASEK.

first image of the corneal stroma immediately after the sub-in basal nerve plexus image at all times in the study.
- Posterior flap: defined as the second anterior stromal sub-layer in presurgery, represented by the corneal stroma image at a depth of 100 μ counting from the epithelium, measured with CMTF curve. Defined as posterior half of the stromal flap during post-surgery, represented by the corneal stroma image immediately prior to the first image of the surgery interface.
- Anterior retroablation: defined in the presurgery as middle stroma, represented by the image located at a depth of 200 μ from the corneal epithelium measured by means of the CMTF curve. Defined in the post-surgery as the first 100μ posterior to the surgical interface,
represented by the corneal stroma image immediately after the last surgical interface image.

- Posterior retroablation: defined at any time of the study as the posterior stroma represented by the images located at 200µ from the corneal endothelium measured with the CMTF curve.
- Posterior stroma: defined as the posterior-most 50 µ adjacent to Descemet's membrane (not visible with confocal microscopy). Represented by the corneal stroma image immediately anterior to the first corneal endothelium image, at any time of the study.

In patients operated with LASEK, the following stromal sub-layers were selected (Fig. 3):
- Anterior stroma: defined as the anterior-most sub-layer of the corneal stroma in the pre- and post-surgery. Represented by the first corneal stroma image immediately posterior to the sub-basal nerve plexus image at any time during the study.
- Middle stroma: defined as the second stroma sub-layer in the pre- and post-surgery, represented by the corneal stroma image at a depth of 150µ from the epithelium, measured with the CMTF curve.
- Posterior stroma: defined as the posterior-most 50µ adjacent to Descemet's membrane (not visible with confocal microscope). Represented by the corneal stroma image immediately anterior to the first corneal endothelium image, at any time during the study.

8. Endothelial cell density (quantitative continuous): defined as the amount of endothelial cells per mm², calculated automatically with confocal microscope software specifically designed for this calculation, utilizing the above described predetermined area and corneal endothelium image.

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**Fig. 2** - Stromal sublayer scheme defined for measuring keratocyte density in LASIK patients. The stroma is located between both membranes standing out from the dark background.

**Fig. 3** - Stromal sublayer scheme defined for measuring keratocyte density in patients operated with LASEK.

* Ablated stroma: portion of the stroma that received Excimer laser ablation, with refractive defect being corrected at the expense of this ablation which reduces the corneal stroma in an amount of µ depending on the magnitude of the treated ametropia. In the post-surgery period this area does not exist and is outlined in gray.
9. Endothelial polymegatism (quantitative continuous): defined as giant cells in the endothelial population. Polymegatism is considered as cellular diameters twice the size of a normal endothelial cell (20 μ), expressed in percentages and calculated automatically by the confocal microscope software which expresses a normal value of below 30%.

10. Endothelial pleomorphism (quantitative continuous): defined as cells having different sizes and loss of normal hexagonal shape in the endothelial population. Expressed in percentages and calculated automatically by the confocal microscope software which expresses a normal value above 60%.

11. Ametropia magnitude (quantitative continuous): defined as the algebraic sum between cylinder and sphere, obtained in dynamic refraction and vision with paralyzed accommodation, expressed in diopters.

12. Sub-basal nerve plexus (qualitative ordinal): defined as the corresponding image described above, measuring with confocal microscope the length of each segment of the visible nerve plexus fibers in an area of 0.0750 mm², identifying the presence or absence of interconnections between them. The following scale of 4 groups was established on the basis of said characteristics:
   - Group 1: No nerve images are seen.
   - Group 2: Short nerves are visible (below 200 mm).
   - Group 3: Longer hours are visible (above 200 mm) without interconnections.
   - Group 4: Long nerves are visible (above 200 mm) with interconnections.

13. Corneal haze thickness (quantitative continuous): defined as the amount of μ comprising continuous corneal haze images in patients operated with LASIK, measured with the CMTF curve from the first stromal image with haze up to the last one, matching peaks in the CMTF curve.

All the surgeries were performed by the author of the study. Surgery was planned with the ORK-CAM application for aspherical treatments. LASIK with pendular microkeratome, flap thickness programmed at 160 μm and residual stromal bed above 300 μm. LASIK with mitomycin C and residual stroma and bed above 400 μm. Both techniques applied to the optic zones of 6.50 mm in all cases.

The treatment was carried out with the ESIRIS Excimer laser (Schwind, Germany). The operations were performed at temperatures of 18–22 °C, relative humidity of 38–42% and layer ablation between 0.530 and 0.580. Both eyes were intervened on the same day. No post-surgery complications arose.

Visits were scheduled at 24 h, 7 days, one month, 3, 6 months and one year after surgery. In each visit the scheduled examinations were carried out (uncorrected visual acuity, corneal topography, dynamic refraction). No post-surgery complications arose up to one year after surgery. Visual acuity without lenses was as previously programmed and remained without variations throughout the year of follow-up in all cases.

The ConfoScan 4 (Nidek, Japan) confocal microscope was used for obtaining and studying in vivo corneal tissue images. The Z-ring for ocular globe fixation with lens 40× was used. The device was programmed in automatic scan mode with central fixation, image acquisition speed of 25 images per second, 550× magnification, lateral resolution of 0.5 μm/pixel, with 350 images per scan at a working distance of 1.98 mm.

Lidocaine (anesthetic eye drops, Quimera) and subsequently Viscoatears (ophthalmic gel) were administered as coupling medium between the cornea and the Z-ring. The lens was advanced up to contacted between the ring and the coupling substance. The lens was aligned with the center of the cornea until the first corneal epithelium images could be seen. Obtained digital images were recorded in automatic mode in a Pentium 4 computer with Windows 2000 for subsequent analysis. Before and after each exam, the lens was cleaned with isopropyl alcohol.

Each obtained image was separated from the adjacent image by 4 μ, a field depth of 25 μm, intensity level from zero to 255, Z-ring pressure of 20%. All the images were taken in the 4 mm around the center of the cornea. None of the subjects experienced visual symptoms or corneal complications during or after the examination.

Confocal microscopy of the cornea was performed in the presurgery, at 7 days, one month, 3, 6 months and one year after surgery. The patients who underwent LASIK did not get this examination at day 7 because it matched the withdrawal of the contact lens which affects the result of the examination. The necessary examinations were made in each patient to obtain means of complete focus quantitative confocal microscopy (CMTF curve) images and scans with the maximum stability as regards pressure applied by the Z-ring with variations below 10%, represented by the yellow curve. The selected images were not modified in brightness or contrast and were encoded for analyzing without knowledge of the post-surgery time, magnitude of treated ametropia or applied surgical technique.

Statistics

The calculations obtained by variables were included in a database in SPSS version 11.5. Descriptive and inferential statistical methods were utilized, expressed in absolute frequencies, relative frequencies and ANOVA for repeated measurements for an adjustment factor of the Bonferroni confidence interval. The correlations between the magnitude of ametropia and morphometric variables were evaluated with bivariate Pearson’s correlation coefficient, excepting the sub-basal nerve plexus variable which was assessed with Spearman’s rho coefficient. The morphometric variables which produced significant correlation above 0.5 with the ametropia magnitude were subjected to multiple linear regression analyses for obtaining the predictive models of morphometric variables in the most advanced post-surgery stage, starting from the ametropia magnitude to be treated and including the presurgery value of the analyzed variable. In the case of nonexistent variables in presurgery, the regression was applied only with the ametropia magnitude.

For each surgical technique, the sample was randomly divided in 2 groups. The regression models were obtained in the first group (estimation), and in the second group the validation thereof was performed. A cross validation was carried out, consisting in calculating 2 correlation coefficients,
the first ($R^2$) between the observed and prognosticating morphometric variable values, in the first group of patients, having values matching $R^2$. The second coefficient ($R^2$) in the second group was obtained between the observed and prognosticating morphometric variables by means of the estimated function in the first group. The reduction index in the cross validation ($R^2 - R^2$) was obtained for each model with values below 0.1 in all cases (Fig. 4).

In order to ensure the validity of said functions, optimal results of the linear regression model assumption were obtained: regression graphs, statistical analysis of residuals and non-colinearity (auto-values, condition indices, tolerance, variance inflation factors). A significance level of 95% was utilized. The results were presented in tables and graphs.

The corresponding flowcharts contained in existing diagnostic and therapeutic protocols in our country were carried out for all patients with ametropia diagnostic. After reassuring each patient about the confidential nature of the study, consents were obtained for inclusion therein.

Results

The multiple linear regression analysis for the morphometric variables is shown below when a significant correlation was found above 0.5 in the ametropia magnitude, also including presurgery values thereof. Residual analysis in all cases exhibited a good adjustment, and reduction indices obtained in the cross validation were below 0.1 in all models, which demonstrated its reliability.

As shown in Table 1, 84.3% of epithelial thickness variations 6 months after LASIK surgery were due to the magnitude of the treated ametropia and the presurgery epithelial thickness. Univariate association sense is preserved: with higher magnitude of ametropia to be treated, post-surgery epithelial growth is larger. In this model, the presurgery value of the variable is more important in accordance with the absolute values of standardized coefficients.

Table 2 shows that 80.1% of pachymetry variations one year after surgery were due to the preoperative pachymetry value and to the magnitude of the ametropia treated with LASIK, with greater influence in the ametropia magnitude model. Univariate association is maintained: with higher ametropia magnitude, lower pachymetry value one year after surgery.

As shown in Tables 3 and 4, linear regression analysis of keratocyte density at both sides of the lamellar section also reached adequate $R^2$ levels. 68.8% of keratocyte density variations in the posterior flap were due to the value of this variable prior to LASIK and to the magnitude of the treated ametropia. In turn, 48.2% of keratocyte density variations in the anterior flap area were due to the value of this variable in the presurgery period and to the magnitude of the treated ametropia.

Diminished keratocyte density one year after surgery in both models is more influenced by the magnitude of ametropia in accordance with the absolute values of the standardized coefficients. Univariate associations are maintained: with higher magnitude of ametropia to be treated, lower density of keratocytes on both sides of the lamellar section.

**Results**

**Table 1**: Sample n=134

- LASIK n=78 (151 eyes)
- LASEK n=56 (111 eyes)

Confocal corneal microscope
Presurgery, at day 7, month 1, 3, 6, 1 year

Random sampling

- Multiple linear regression (predictive histological variable models)
- Obtention of assumed regression models
- Correlation between observed values of histological variables and prognosticated values (RIa)

Reduction index per model: $R_{1/b} - R_{1/a} < 0.12$

**Fig. 4 – Flowchart of the study.**

**Tables 5-7** illustrate the linear regression functions in LASEK. 90% of corneal pachymetry variations one year after LASEK were due to the value of this variable in the presurgery period and to the magnitude of treated ametropia, with presurgery pachymetry having greater influence.

Overall, 53% of keratocyte density variations in anterior stroma one year after LASEK were due to the value of this variable in the presurgery period and to the magnitude of treated ametropia. In this case, ametropia magnitude has greater importance in the model.

The obtained $R^2$ value is not optimal in the case of corneal haze after LASEK. Only 39% of variations are dependent on the magnitude of treated ametropia. Univariate associations described above in LASEK are maintained.

Discussion

Ametropia magnitude has a directly proportional influence on the depth of ablation and the treatment time with Excimer laser, according to the Munnerlyn formula: $T = 52D/3$, where $T$ is the central ablation depth ($\mu m$), $S$ is the optic area diameter and $D$ is the magnitude of the ametropia to be treated, measured in diopters.11,12
Table 1 – Multiple linear regression: epithelium thickness 6 months after LASIK.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonstandardized coefficients</th>
<th>Standardized coefficients</th>
<th>CI 95%</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>TE</td>
<td>LL</td>
<td>HL</td>
</tr>
<tr>
<td>Constant</td>
<td>4.331</td>
<td>1.901</td>
<td>0.605</td>
<td>8.057</td>
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<tr>
<td>MA</td>
<td>0.723</td>
<td>0.073</td>
<td>0.580</td>
<td>0.866</td>
</tr>
<tr>
<td>pET</td>
<td>0.842</td>
<td>0.054</td>
<td>0.736</td>
<td>0.948</td>
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<td>$R^2_s = 0.017$</td>
<td>ET = $4.331 \times 0.723 \times MA + 0.842 \times pET$</td>
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<tr>
<td>Validation</td>
<td>$R^2_s - R^2 = 0.017$</td>
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<tr>
<td>Function</td>
<td>PAQ = $62.867 - 10.560 \times MA + 0.863 \times pET$</td>
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<td></td>
</tr>
</tbody>
</table>

B: nonstandardized partial regression coefficient; TE: typical error; ET: epithelial thickness at 6 months; pET: presurgery epithelial thickness; CI: confidence interval; LL: lower limit; HL: higher limit; MA: magnitude of ametropia.

Table 2 – Multiple linear regression: pachymetry one year after LASIK.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-standardized coefficients</th>
<th>Standardized coefficients</th>
<th>CI 95%</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>TE</td>
<td>LL</td>
<td>HL</td>
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<td>142.2</td>
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<td>MA</td>
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<td>−16.423</td>
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<td>pPAQ</td>
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<td>0.071</td>
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<td>PAQ = $62.867 - 10.560 \times MA + 0.863 \times pPAQ$</td>
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<tr>
<td>Validation</td>
<td>$R^2_s - R^2 = 0.013$</td>
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<tr>
<td>Function</td>
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</tbody>
</table>

B: nonstandardized partial regression coefficient; TE: typical error; CI: confidence interval; LL: lower limit; HL: higher limit; MA: magnitude of ametropia; PAQ: pachymetry at one year; pPAQ: presurgery pachymetry.

Table 3 – Multiple linear regression: density of flap keratocytes one year after LASIK.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-standardized coefficients</th>
<th>Standardized coefficients</th>
<th>CI 95%</th>
<th>p</th>
</tr>
</thead>
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<td></td>
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<tr>
<td>(Q) FPp</td>
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<td>$R = 0.829$. $R^2 = 0.688$. Corrected $R^2 = 0.679$</td>
<td>$R^2_s = 0.006$</td>
<td>(Q) FP = $20.861 - 1.209 \times MA + 0.352 \times (Q) FPp$</td>
<td></td>
</tr>
<tr>
<td>Validation</td>
<td>$R^2_s - R^2 = 0.006$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Function</td>
<td>(Q) FP = $20.861 - 1.209 \times MA + 0.352 \times (Q) FPp$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B: nonstandardized partial regression coefficient; TE: typical error; CI: confidence interval; LL: lower limit; HL: higher limit; MA: magnitude of ametropia. (Q) FP: density of posterior flap kerocytes after one year; (Q) FPp: presurgery posterior flap kerocyte density.

The theoretical–mathematical model developed by Huang simulates epithelial migration steps subsequent to laser corneal refractive surgery, which predicts the central epithelial thickness after myopic ablation. Thickness changes that remain subsequently are due to tissue hyperplasia, which were documented up to 7 years after LASIK. On the other hand, several studies have pointed out the influence of said epithelial hyperplasia in refractive changes.

Table 4 – Multiple linear regression: density of anterior retroablation keratocytes one year after LASIK.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-standardized coefficients</th>
<th>Standardized coefficients</th>
<th>CI 95%</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>TE</td>
<td>LL</td>
<td>HL</td>
</tr>
<tr>
<td>Constant</td>
<td>8.941</td>
<td>10.672</td>
<td>−11.976</td>
<td>29.858</td>
</tr>
<tr>
<td>MA</td>
<td>−0.952</td>
<td>0.213</td>
<td>−1.369</td>
<td>−0.535</td>
</tr>
<tr>
<td>(Q) RAp</td>
<td>0.724</td>
<td>0.368</td>
<td>0.003</td>
<td>1.445</td>
</tr>
<tr>
<td>Model</td>
<td>$R = 0.694$. $R^2 = 0.482$. Corrected $R^2 = 0.467$</td>
<td>$R^2_s = 0.032$</td>
<td>(Q) RA = $8.941 - 0.952 \times MA + 0.724 \times (Q) RAp$</td>
<td></td>
</tr>
<tr>
<td>Validation</td>
<td>$R^2_s - R^2 = 0.032$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Function</td>
<td>(Q) RA = $8.941 - 0.952 \times MA + 0.724 \times (Q) RAp$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B: nonstandardized partial regression coefficient; TE: typical error; CI: confidence interval; LL: lower limit; HL: higher limit; MA: magnitude of ametropia; (Q) RA: density of anterior retro-ablation keratocytes after one year; (Q) RAp: density of anterior retro-ablation keratinocytes.
after LASEK. The epithelial thickness changes between pre- and post-surgery have an impact on the refractive power of epithelium. The central epithelium thickness increase described in the corneal angle could explain the myopia described in early stages after LASEK. An increase of 10 μm in epithelium thickness causes 1 diopter of regression, while mechanical and therapeutic factors control epithelial hyperplasia. Corneal epithelium modifications play a role in the final refractive results. However, refractive changes have taken place between one month and one year when the epithelium thickness is already stabilized, which gives rise to the idea that epithelial changes are not the only factor of influence in myopic regression.

An additional factor of influence in said variable is the pharmacological factor. Instilled eyedrops (steroids, tear substitutes and antibiotics), particularly prednisolone, chloramphenicol and artificial tears, reach the pre-epithelial lacrimal film penetrating absolutely through the corneal epithelium due to simple diffusion, even more so if the medication is liposoluble as is the case of the majority of products utilized in this type of procedure.

The corneal epithelium constitutes a continuous layer of plasmatic membranes due to the union of their cells by means of Zonula Occludens. Lipophilic pharmaceuticals easily pass through epithelium as their plasmatic membranes are comprised of phospholipids. As the corneal epithelium has over two thirds of the cornea plasmatic membranes, this is the place where the majority of lipophilic drugs are deposited. In addition, the high frequency of application of eyedrops in the first post-surgery month is another influence in epithelium thickness. This reinforces the pharmacological factor, which is present in the LASEK technique in which the epithelium is whole 24 h after the procedure.

Corneal regulation mechanisms enable corneal homeostasis and therefore the attenuation of the consequences induced by the above-mentioned factors, which

| Table 5 – Multiple linear regression: pachymetry one year after LASEK. |
|-----------------|----------------|-----------------|----------|----------|
| Variable        | Non-standardized coefficients | Standardized coefficients | CI 95%   | p        |
|                 | B               | TE              | LL       | HL       |
| Constant        | 52.216          | 26.352          | 0.5661   | 103.867  | 0.046   |
| MA              | −10.031         | 0.628           | −11.262  | −8.800   | <0.0001 |
| pPAQ            | 0.871           | 0.044           | 0.785    | 0.957    | <0.0001 |
| Model           | R = 0.952. R² = 0.904. Corrected R² = 0.893 |
| Validation      | R² = R² = 0.012 |
| Function        | PAQ = 52.216 − 10.031 × MA + 0.871 × pPAQ |

B: nonstandardized partial regression coefficient; TE: typical error; CI: confidence interval; LL: lower limit; HL: higher limit; MA: magnitude of ametropia; pPAQ: pachymetry at one year; pPAQ: presurgery pachymetry.

| Table 6 – Multiple linear regression: density of anterior stromal keratocytes one year after LASEK. |
|-----------------|----------------|-----------------|----------|----------|
| Variable        | Non-standardized coefficients | Standardized coefficients | CI 95%   | p        |
|                 | B               | TE              | LL       | HL       |
| Constant        | 21.018          | 5.219           | 10.789   | 31.247   | <0.0001 |
| MA              | −1.207          | 0.064           | −1.332   | −1.082   | <0.0001 |
| (Q) EAp         | 0.366           | 0.194           | −0.014   | 0.746    | 0.044   |
| Model           | R = 0.730. R² = 0.533. Corrected R² = 0.529 |
| Validation      | R² = R² = 0.016 |
| Function        | (Q) EA = 21.018 − 1.207 × MA + 0.366 × (Q) EAp |

B: nonstandardized partial regression coefficient; TE: typical error; CI: confidence interval; LL: lower limit; HL: higher limit; MA: magnitude of ametropia; (Q) EA: density of anterior stroma keratocytes at one year; (Q) EAp: presurgery density of anterior stroma keratocytes.

| Table 7 – Multiple linear regression: corneal haze thickness one year after LASEK. |
|-----------------|----------------|-----------------|----------|----------|
| Variable        | Non-standardized coefficients | Standardized coefficients | CI 95%   | p        |
|                 | B               | TE              | LL       | HL       |
| Constant        | 19.886          | 8.121           | 3.969    | 35.803   | <0.034  |
| MA              | 0.753           | 0.409           | −0.049   | 1.555    | <0.0001 |
| Model           | R = 0.628. R² = 0.394. Corrected R² = 0.391 |
| Validation      | R² = R² = 0.008 |
| Function        | Gh = 19.886 − 0.753 × MA |

B: nonstandardized partial regression coefficient; TE: typical error; Gh: haze thickness; CI: confidence interval; LL: lower limit; HL: higher limit; MA: magnitude of ametropia.
is verified in the progressive epithelial thickness reduction up to one year post-surgery with levels similar to those of the presurgery period in LASIK. In addition, the patients of the present study maintained their uncorrected visual acuity within constant values, independently of the epithelium thickness changes in the post-surgery period. This evidences the lack of influence of these modifications in the visual result.28

After LASEK, the corneal epithelium begins a cicatriziation process which lasts 7 days, at which point contact lenses are withdrawn. The preservation of corneal epithelium and its reposition during the trans-surgery period with the greatest possible perfection in what concerns regular edges, absence of erosions and application of the minimum necessary alcohol time, come to bear on a correct and quick cicatriziation of the edges, which explains the lack of variations in epithelium thickness one month after surgery.

The differences between authors are mainly due to the confocal microscope model, objective lenses, axial and lateral resolutions and working distances, among others, which significantly influence the contrast of images as well as resolution and subsequent analysis.

The present study did not observe manifest refractive regression because the magnitude of treated ametropia did not exceed 8 diopters of ablation and therefore the effect of the Excimer laser on epithelial thickness was lower and with less duration than in other studies29,30 which have reported ablations of up to 12 diopters, which undoubtedly exert a greater effect on corneal tissue.

Corneal stroma is the sublayer which undergoes more changes because it directly receives Excimer laser action. It is precisely in the shaping of the stroma where corneal curvature is achieved with a view to the final refractive result of the patient.

Diminished pachymetry in the post-surgery period is due to the loss of central stromal tissue that is produced in order to achieve the desired refractive defect.31–34 The negative direct correlation with the magnitude of ametropia matches the statements by Munnerlyn in what concerns ablation depth and its relationship with this variable.35–37 The predicted actual pachymetry value one year after corneal refractive surgery, based on the magnitude of the treated ametropia and of the presurgery pachymetry measured with microscopy, enable an optimum selection of patients for this surgery. This is a factor which determines the surgical success of these procedures as well as their long-term safety. In addition, obtaining this pachymetry value one year in advance ensures optimal pachymetry after treatment, including the possibility of new refractive treatment if necessary. On the other hand, the fact of knowing that this variable on the basis of in vivo presurgery pachymetry influences the selection of the type of treatment to be applied,38 where the selection of the surface laser treatment could enhance protective pachymetry at year one.39

The pachymetry values obtained are applied to the patient with greater precision than the usually applied ultrasound pachymetry techniques. The values obtained in vivo through microscopy provide an almost perfect exactitude vis-à-vis actual values, analyzing each patient with greater precision, reliability and predictability of expected morphological parameters at year one post-surgery.

The direct negative correlation between diminished keratoctye density and higher ametropia magnitude to be treated is evident in both surgical techniques. With deeper ablation, more stromal tissue is removed and the photo-ablation factor is greater in what concerns time of action on the corneal stroma, with its morphometric consequences.

The photo-ablation action time factor is related to the frequency of application of the shots, specifically with the corneal stroma hydration changes that take place with time. Longer treatments could adversely effect tissue hydration. The photo-ablation action time is inversely proportional to the corneal hydration level, i.e., the corneal loses hydration together with the increase of laser application time. Accordingly, more corneal tissue is removed, which explains the greater photo-ablation effect on the keratocyte population with greater ametropia magnitudes, and therefore this influences the density reduction of this cellular line.22,31,40

The clinical significance of this gradual loss of keratocytes, as well as the density required of these cells to maintain corneal transparency, is unknown.41 It is important to determine this parameter to understand the behavior of these collagen- and proteoglycans-producing cells which are necessary to maintain tissue, as its deficiency for many years could affect corneal transparency and curvature. Accordingly, the importance of studying this before and after corneal surgeries acquires increasing relevance.

In the patients in whom we have verified diminished keratocyte density, refraining from modifying this variable in deep stromal layers is a safety element of the applied surgical technique which guarantees long term corneal health. The authors consider that significant keratocyte population reductions in other studies in deep stromal layers is due to the greater magnitude of treated ametropia with the ensuing increased ablation depth.

Even though corneal changes have not been reported in a post-surgery visual acuity in the presence of diminished keratocyte density, the minimum possible alteration of stromal homeostasis should become the fundamental premise of all refractive defect surgeons, with a view to promote optimum visual acuity and corneal function for the entire lifetime of the patient. Among other factors, this depends on the structure and functional integrity of the fundamental corneal stroma cell, i.e., keratocytes. Accordingly, in patients with ametropia magnitudes in the area of 8 diopters, regardless of the baseline corneal pachymetry value, it is prudent to select a surface treatment with small a histological repercussion in the lower corneal stroma sub-layers where the authors have verified that keratocyte reduction is lower.

The corneal haze values obtained after LASEK demonstrates that, regardless of the magnitude of treated ametropia in all cases, there is some degree of haze in the in vivo microscopic study even though it is not detectable during ophthalmological examination. In addition, the haze thickness increases in parallel with the treated ametropia magnitude even though, as statistically demonstrated herein, this variable only partly explains the haze thickness, and this confirms the results of studies which mention other cicatricial response factors which are particular to each patient.42–45 The obtained predictive models reveal that variations in the morphometric variables one year after treatment largely depend on
presurgery values and the magnitude of ametropia to be treated. These equations constitute tools to be taken into account as selection criteria for candidates to excimer laser surgery for treating ametropia as well as the optimal choice of the surgical technique.

Conflict of interest

None declared.

REFERENCES


