Objective Monitoring of Corneal Backward Light Scattering After Femtosecond Laser-assisted LASIK

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ABSTRACT

PURPOSE: To investigate the changes in corneal backward light scattering, as measured by a rotating Scheimpflug camera with automated corneal densitometry software, in eyes treated with femtosecond laser-assisted LASIK (FS-LASIK).

METHODS: The cornea was examined preoperatively and postoperatively at 1 day, 1 week, and 1, 3, and 6 months in 23 patients who underwent myopic FS-LASIK. Local analysis of corneal backscatter was performed on four concentric radial zones across a 12-mm diameter (0 to 2, 2 to 6, 6 to 10, and 10 to 12 mm) and at a different corneal depth (anterior 120 µm, central and posterior 60 µm).

RESULTS: A statistically significant increase in corneal backward light scattering (P < .0001) was detected within the central 10 mm of the anterior cornea. The increase in corneal densitometry was gradually reversed over 6 months. The difference compared to preoperative values was no longer statistically significant at 3 and 6 months after surgery in the central cornea, whereas it remained significant in the mid-peripheral annulus (ranging from 6 to 10 mm), where the flap edge was located.

CONCLUSIONS: FS-LASIK is followed by an increase in corneal backward light scattering during the early postoperative period that returns to baseline by 3 months. Whereas the increase in corneal densitometry at the flap edge location can be related to a scarring reaction, the explanation for such an increase in the central anterior cornea remains speculative.

for a single corneal scan.\textsuperscript{10,11} Alternatively, purpose-written software designed to process and analyze the exported Scheimpflug images are needed.\textsuperscript{12} These methods have some limitations because they evaluate only one or two sections instead of the whole cornea. They are time-consuming and cannot be applied in the clinical routine, and they also may not be commercially available.\textsuperscript{12}

Recently, a commercial rotating Scheimpflug camera (Pentacam; Oculus Optikgeräte, Wetzlar, Germany) has been implemented with a software application that generates a fully automated corneal densitometry map.\textsuperscript{13,14} In this study, we aimed to investigate the changes in corneal backward light scattering in eyes that had undergone femtosecond laser-assisted LASIK (FS-LASIK) for the correction of myopia and myopic astigmatism during the 6-month postoperative follow-up.

**PATIENTS AND METHODS**

A prospective analysis was done on a consecutive sample of patients who had FS-LASIK in a private practice between September and December 2013. Patients underwent Scheimpflug imaging of the cornea preoperatively and postoperatively at 1 day, 1 week, and 1, 3, and 6 months. They were asked to stop using soft and rigid contact lenses at least 15 and 30 days, respectively, before the preoperative examination. Exclusion criteria were any intraoperative or postoperative complication and any preexisting corneal pathology. Before being included in the study, all patients were informed that preoperative and postoperative measurements could be used for scientific purposes and gave their written consent. The study methods adhered to the tenets of the Declaration of Helsinki for the use of human participants in biomedical research and were approved by the local ethics committee.

**SURGICAL TECHNIQUE**

The patients enrolled in the study underwent uncomplicated primary bilateral FS-LASIK using the same refractive surgery platform (WaveLight FS200 femtosecond laser and WaveLight EX500 excimer laser; Alcon Laboratories, Inc., Fort Worth, TX). All surgeries were performed by one surgeon (GS). The flap thickness was set at 120 µm and the planned flap diameter was 9 mm. Pulse energy for bed cut and side cut ranged between 0.66 and 0.70 µJ. After surgery, patients were prescribed 0.3% netilmicin and 0.1% dexamethasone (Netildex; SIFI, Catania, Italy) to be taken four times a day for the first week and preservative-free artificial tears to be taken every hour for the first day and then slowly tapered off during the following 3 months.

**MEASUREMENT TECHNIQUE**

During Scheimpflug scan acquisition, participants were seated comfortably and alignment was achieved using the table height adjustment, forehead strap, and chin rest. They were instructed to keep both eyes open and look directly at the fixation target centered in the scanning-slit light for the duration of the scan. Scans were repeated if the quality specification provided by the instrument was other than “OK.”

The Pentacam (software version 1.20r10) takes a series of 25 images (1,003 × 520 pixels) over different meridians with a uniform blue light source. The device automatically locates the corneal apex and analyzes an area around it with a diameter of 12 mm. The output is expressed in grayscale units, which are calibrated by proprietary software from a minimum light scatter of 0 (maximum transparency) to a maximum light scatter of 100 (minimum transparency). For the purposes of local densitometry analysis, the 12-mm diameter area was divided into four concentric radial zones. The first, the central zone, was 2 mm in diameter and centered on the apex. The second zone was an annulus extending from 2 mm to a 6-mm diameter circle. The third zone was an annulus extending from 6 to 10 mm. The final zone extended from 10 mm to a 12-mm diameter circle. These topographical zones are available preset in the software. The output can also be divided into anterior, central, and posterior layers based on corneal depth: the anterior layer corresponds to the anterior 120 µm and the posterior layer to the most posterior 60 µm of the cornea. The central corneal layer has no fixed thickness, but is defined by subtraction of the anterior and posterior layers from the total thickness. Furthermore, the layer densitometry may be calculated for each of the concentric radial zones, which enables a more detailed analysis based on both area and depth.

The following values were investigated in the current study: 0 to 2 mm anterior corneal 120 µm, 2 to 6 mm anterior corneal 120 µm, 6 to 10 mm anterior corneal 120 µm, 10 to 12 mm anterior corneal 120 µm, total (0 to 12 mm) anterior corneal 120 µm, total (0 to 12 mm) central corneal stroma, total (0 to 12 mm) posterior corneal 60 µm, and total (0 to 12 mm) anterior + central + posterior cornea.

Given that corneal edema has been related to increased scattering,\textsuperscript{1,15} we also investigated the minimum corneal thickness at each postoperative examination.

**STATISTICAL ANALYSIS**

Because densitometry data were not normally distributed, as determined by the Kolmogorov–Smirnov test, the mean values of corneal densitometry from the preoperative examination to the 6-month follow-up were compared using the Friedman test (non-parametric...
TABLE 1
Corneal Densitometry Before and After Femtosecond Laser-assisted LASIK (Mean ± SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Preoperative</th>
<th>1 Day</th>
<th>1 Week</th>
<th>1 Month</th>
<th>3 Months</th>
<th>6 Months</th>
<th>Friedman’s Test P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 2 mm anterior 120 µm</td>
<td>23.05 ± 1.62</td>
<td>26.12± 2.69</td>
<td>25.03± 1.26</td>
<td>24.29± 1.36</td>
<td>23.67± 1.62</td>
<td>23.40± 1.58</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>2 to 6 mm anterior 120 µm</td>
<td>20.88 ± 1.53</td>
<td>23.85± 2.55</td>
<td>22.94± 1.19</td>
<td>22.23± 1.20</td>
<td>21.80± 1.62</td>
<td>21.65± 1.34</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>6 to 10 mm anterior 120 µm</td>
<td>20.51 ± 3.34</td>
<td>22.90± 4.37</td>
<td>22.60± 3.74</td>
<td>22.43± 3.72</td>
<td>22.33± 4.09</td>
<td>22.24± 3.51</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>10 to 12 mm anterior 120 µm</td>
<td>29.46 ± 7.37</td>
<td>26.16± 7.57</td>
<td>27.39± 6.78</td>
<td>27.13± 6.80</td>
<td>26.85± 5.78</td>
<td>27.28± 5.79</td>
<td>NS (.1471)</td>
</tr>
<tr>
<td>0 to 12 mm anterior 120 µm</td>
<td>22.57 ± 2.19</td>
<td>24.33± 3.40</td>
<td>23.93± 2.39</td>
<td>23.47± 2.34</td>
<td>23.14± 2.59</td>
<td>23.07± 2.28</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>0 to 12 mm central</td>
<td>14.70 ± 1.74</td>
<td>15.02± 2.00</td>
<td>15.13± 1.70</td>
<td>15.15± 1.73</td>
<td>15.21± 1.78</td>
<td>15.33± 1.52</td>
<td>NS (.1032)</td>
</tr>
<tr>
<td>0 to 12 mm posterior 60 µm</td>
<td>12.89 ± 1.81</td>
<td>12.85± 1.88</td>
<td>13.42± 1.86</td>
<td>13.37± 1.81</td>
<td>13.47± 1.91</td>
<td>13.55± 1.71</td>
<td>NS (.6543)</td>
</tr>
<tr>
<td>0 to 12 mm anterior, central, and posterior</td>
<td>16.66 ± 1.64</td>
<td>17.39± 2.35</td>
<td>17.41± 1.77</td>
<td>17.26± 1.85</td>
<td>17.22± 1.93</td>
<td>17.26± 1.71</td>
<td>.0325</td>
</tr>
</tbody>
</table>

SD = standard deviation; NS = not significant
*P < .001 in comparison to the preoperative value, Dunn’s posttest.
**P < .05 in comparison to the preoperative value, Dunn’s posttest.
***P < .01 in comparison to the preoperative value, Dunn’s posttest.

repeated measures of analysis of variance (ANOVA)]. Dunn’s posttest was performed to compare each postoperative densitometry measurement to the preoperative value. Repeated ANOVA (with the Bonferroni posttest) was used to compare mean values of corneal pachymetry. A P value less than .05 was considered statistically significant. All statistical tests were performed using GraphPad InStat (version 3a) for Macintosh (GraphPad Software, San Diego, CA). Because all patients had bilateral surgery, only one randomly chosen eye was considered for statistical analysis.

RESULTS

Twenty-three patients (mean age: 35 ± 8.4 years; range: 21 to 59 years) were enrolled. Preoperative corrected distance visual acuity was 20/20 or better in all eyes. The mean attempted refractive change was -3.20 ± 2.00 diopters (D) (range: -1.00 to -6.50 D). The mean planed ablation depth was 55.1 ±18.6 µm. All eyes had a postoperative uncorrected distance visual acuity of 20/20 or better and a manifest refraction within ±0.50 D of emmetropia from the first postoperative week up to the last follow-up. No signs of central superficial punctate keratopathy were observed during the follow-up.

Table 1 shows that a statistically significant increase in corneal backward light scattering was detected within the central 10 mm of the anterior cornea from postoperative day 1. Corneal backward light scattering gradually decreased over the following 6 months. The difference compared to the preoperative values was no longer statistically significant at 3 and 6 months after surgery in the central 6 mm of the anterior cornea, whereas it remained significant in the annulus (ranging from 6 to 10 mm, where the flap edge is located). No statistically significant changes were observed in the peripheral anterior cornea between 10 and 12 mm or in the central and posterior corneal layers. A statistically significant increase in corneal densitometry was also observed in the first week for measurements including the whole anterior corneal layer (0 to 12 mm) and the whole cornea (0 to 12 mm from anterior to posterior stroma).

At 1 month postoperatively, linear regression revealed a statistically significant correlation between the planned ablation and the increase in corneal backward light scattering in the 2- to 6-mm anterior corneal zone ($R^2 = 0.1973, P = .0383$) and in the 6- to 10-mm anterior corneal zone ($R^2 = 0.3197, P = .0661$). Such a relationship was not statistically significant for the remaining zones and time intervals.

Figure 1 shows one example of the increase in corneal backward light scattering.

Postoperatively, the minimum corneal thickness was 493.5 ± 51.2 µm on day 1. Then it decreased to 479.3 ± 46.9 µm at 1 week, and slightly increased to 480.9 ± 44.1 µm at 1 month, 483.0 ± 45.6 µm at 3 months, and 485.9 ± 42.8 µm at 6 months. Repeated measures ANOVA revealed a statistically significant difference ($P < .0001$). However, posttest analysis detected a statistically significant difference only between day 1 and each postoperative control, whereas from 1 week to 6 months the mean values remained substantially unchanged.

DISCUSSION

Our data reveal that FS-LASIK for myopia increases backward light scattering in the anterior layers of the cornea during the early postoperative period. Corneal scattering gradually decreases over time and returns...
to the preoperative values by the third postoperative month over the 6-mm central area. However, corneal densitometry values remain higher than preoperative values even at 6 months postoperatively in the mid-peripheral ring (6 to 10 mm), where the flap edge is located. No statistically significant changes were observed in the peripheral anterior cornea (10 to 12 mm) or in the central and posterior corneal layers.

To our knowledge, this is the first study to reveal that FS-LASIK increases corneal light backscattering, as measured by a rotating Scheimpflug camera, in the early postoperative period. Previously, Fares et al. did not find any difference between the preoperative and 12-month mean corneal densitometry (although their study was limited by the fact that the authors investigated the corneal densitometry along only four axes and the analyzed area was manually delimited by a caliper, due to the unavailability of current densitometry software). Their data are nonetheless in good agreement with ours, because we observed that the increase in densitometry values was no longer evident across the central cornea at 3 months. Our data also confirm a previous study by Patel et al., who used a custom scatterometer to measure corneal light backscattering and found increased values in the anterior and middle thirds of the cornea at 1 and 3 months after FS-LASIK. In the mid-peripheral cornea, where the flap edge is created, the increase in cornea densitometry is likely to depend on the fibrotic wound repair and subsequent scarring reaction that can be detected at the slit lamp.

In the central cornea, the explanation for increased densitometry is more difficult and is in contrast with the clinical appearance at the slit lamp, where no opacities were detected. At least five mechanisms may be involved: (1) surgery-related mechanical damage of the corneal epithelium, (2) dry eye, (3) corneal edema, (4) deposition of particles in the interface, and (5) laser-related inflammation. Whereas the first mechanism is likely to have a short-term influence and should not affect the results at 1 week and later, the remaining ones may play a role for a longer time.

Ocular surface dryness has been shown to induce greater corneal backward light scattering when central superficial punctate keratopathy is present. However, central superficial punctate keratopathy was not detected in our sample, so we can rule out dry eye as a causative factor.

Temporary corneal edema may be another pathophysiological explanation for increased light backscattering due to an irregular arrangement of collagen fibrils and increased corneal thickness. In our study, pachymetry data suggested that FS-LASIK induced a slight corneal edema on the first postoperative day only. Thereafter, the corneal thickness decreased and did not show any statistically significant change from the first week to sixth month in comparison with the preoperative state. Therefore, corneal edema likely did not influence central corneal backward scattering, at least when this was detected at 1 week and 1 month.

**Figure 1.** An example of corneal densitometry (left) preoperatively and (right) 3 months after femtosecond laser-assisted LASIK showing an increase in backward light scattering in the central corneal area and along the flap edge.
Light backscattering may be related to interface particles, which may act as tiny mirrors causing random reflection as a result of random orientation relative to the incident beam. No such particles were observed at the slit lamp in our patients, but they might have been too small to be detected and thus cannot be definitely ruled out as a causative factor.

Finally, corneal inflammation may be considered one of the most likely mechanisms to explain increased backward light scattering. Although both excimer and femtosecond lasers may be involved, a case report by Kitzmann et al. suggests that femtosecond laser alone is sufficient to induce corneal inflammation and haze and thus contribute to the increase in corneal densitometry. After uneventful LASIK, the inflammation of the flap interface has usually been defined as diffuse lamellar keratitis, first described by Smith and Maloney in 1998 as a granular white cellular infiltrate. Diffuse lamellar keratitis has also been reported after FS-LASIK, at an even higher incidence than with manual microkeratomes. Although in the current study no patient revealed any sign of diffuse lamellar keratitis at the slit-lamp examination during any postoperative visit, we cannot rule out that the increased light backscattering was due to keratocyte activation. This hypothesis is supported by histopathology studies, which have shown that FS-LASIK triggers cellular necrosis and keratocyte apoptosis at both the flap edge and central interface.

Confocal microscopy has shown activated keratocytes and haze at the flap interface at postoperative day 1 in a patient who had undergone femtosecond laser flap creation, but not flap separation or excimer laser treatment. In the same patient, keratocyte activation gradually resolved over 2 months. Three months postoperatively, confocal microscopy revealed that keratocyte activation occurs at the flap interface, may extend up to 20 µm beyond the interface plane, and is associated with higher interface reflectivity. In the central cornea, such changes cannot be observed at the slit lamp, but may be identified by Scheimpflug imaging.

The measured femtosecond laser energy level in our sample (ranging between 0.66 and 0.7 µJ) was close to that of the low level energy group analyzed by de Medeiros et al., who investigated the effects of a 60-KHz femtosecond laser in rabbit eyes and found that even 0.5 µJ induced necrosis and inflammation at the flap interface. However, the energy was lower than that reported by the previously cited studies analyzing keratocyte activation by confocal microscopy in humans, where the energy ranged between 1.3 and 2.5 µJ. It should be noted that the above-mentioned studies were all based on the IntraLase laser (Abbott Medical Optics, Santa Ana, CA), whereas our patients had been operated on with the FS200, whose effects on the cornea have never been investigated by means of confocal microscopy or histopathology.

One month after surgery, we also detected a correlation between the planned ablation depth and the increase in corneal densitometry. This observation suggests that the excimer laser also plays a role in the increase of corneal light backscattering after FS-LASIK, probably due to the higher degree of corneal inflammation induced by the higher amount of energy required by deeper ablations. A similar result was described almost 20 years ago by Braunstein et al., who found that after photorefractive keratectomy the light-scattering index was significantly greater in eyes with an ablation depth of more than 80 µm than in those with an ablation depth of 80 µm or less at 1 and 3 months.

A limitation of this study is that we did not correlate the corneal densitometry measurements to other optical properties of the cornea, such as higher order aberrations, or to visual quality tested by contrast sensitivity. Further studies are needed to address these issues and investigate whether densitometry values may be influenced by any medical treatment.

Scheimpflug imaging shows that during the first 6 postoperative months, FS-LASIK induces an early increase in corneal backward light scattering and densitometry values. These changes can be observed in the anterior corneal layers, gradually decrease, and the densitometry returns to the preoperative values in the central 6 mm within 3 months, but the increase persists in the area corresponding to the flap edge for up to 6 months. Possible causative factors include interface particles and corneal inflammation that are not detectable at the slit-lamp examination.

**AUTHOR CONTRIBUTIONS**

Study concept and design (GS, JH, ML, SS, DS-L, SV, PD); data collection (GS, SV); analysis and interpretation of data (GS, ML, SS, DS-L, SV, PD); writing the manuscript (GS); critical revision of the manuscript (GS, JH, ML, SS, DS-L, SV, PD); supervision (PD)

**REFERENCES**


