# Retinal nerve fiber layer measurements using scanning laser polarimetry after photorefractive keratectomy

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PURPOSE. *To assess the effects of corneal depth changes on retinal nerve fiber layer (RNFL) postoperative measurements in myopic patients who had undergone photorefractive keratectomy (PRK).*

METHODS. *A total of 120 myopic patients underwent PRK for myopia (range -2 to -10 diopters) and were divided into three groups according to their myopic correction: lower than 3 diopters (low myopia group), between 3 and 6 diopters (medium myopia group), over 6 diopters (high myopia group). RNFL parameters were evaluated preoperatively and 5 days, 3 months and 6 months after surgery, using a GDx NFA II scanning laser ophthalmoscope.*

RESULTS. *Significant changes were seen in the symmetry, superior maximum, and average thickness, comparing baseline with six-month measurements (p=0.008, 0.027, 0.015 respectively). Dividing the sample according to attempted myopic correction, it was found that mean postoperative RNFL thickness was significantly lower after PRK only in the high myopia group. Mean RNFL thickness did not change with time (p = 0.884). Ablation depth was correlated with a change in RNFL thickness by the sixth postoperative month for each group. These variables were significantly related only in the high myopia group (p=0.003).*

CONCLUSIONS. *As polarised light penetrates the ablation area, morphological and functional changes might affect Gdx NFA II measurements. It must always be borne in mind that RNFL thickness can decrease either in cases of glaucoma or after PRK for high myopia, so close attention must be paid to interpreting these measurements in patients who have undergone PRK. (Eur J Ophthalmol 2000; 10: 137-43)*

KEY WORDS. *Retinal nerve fiber layer, Scanning laser polarimetry, PRK*

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## INTRODUCTION

Several methods have been reported for clinical evaluation of the peripapillary retinal nerve fiber layer (RNFL) (1, 2). Red-free ophthalmoscopy or photographic techniques with shorter (495 nm) wavelengths detect local changes and diffuse defects but these subjective techniques do not provide quantitative information (3,4).

A new computerised technique, scanning laser polarimetry, has recently been introduced. This method is based on the theoretical assumption that ocular tissues are birefringent and therefore modify the state of polarisation of a near-infrared diode laser beam that passes through. This change in the state of polarisation, called retardation, is found to be proportional to the thickness of the anterior segment and of the RNFL. Assuming that corneal polarization is mostly compensated by GDx NFA optical devices, scanning laser polarimetry provides indirect measurements of the morphology and thickness of the RNFL (4-6).

Excimer laser photorefractive keratectomy (PRK) reprofiles the anterior corneal surface and modifies corneal power (7,8). As corneal ablation depth is proportion-

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al to the attempted myopic correction, the aim of our study was to evaluate whether corneal depth changes affected RNFL postoperative measurements in myopic patients (8-10).

## METHODS

*Preoperative patient selection:* Between September and November 1998, 151 patients applied for excimer laser surgery for myopia at the Department of Ophthalmology, University of Bari, Italy. They entered this non-randomised, comparative study and gave informed consent. Inclusion criteria were: age 18 to 50 years, stable refraction for at least two years, regular astigmatism lower than 3 diopters (D), best corrected visual acuity higher than 0.2 reported on the logarithm of the minimum angle of resolution (LogMAR) scale (11). Patients with a history of corneal disease (infectious, inflammatory, degenerative), ocular hypertension (untreated IOP greater than 22 mmHg with normal-appearing optic nerves and visual field), lens opacities, previous refractive surgical treatment, wound healing abnormalities (e.g. keloids) were excluded from the study.

We planned corrections ranging from 2 D to 10 D, because the nerve fiber analyzer used in this study (GDx-NFA II, Laser Diagnostic Technologies Inc., San Diego, CA, USA, software version 1.0.09) supports a device which compensate ametropia defects ranging from -10 to + 10 D. Patients whose myopic corrections were lower than 3 D were included in the "low myopia" group, those with corrections ranging from 3 to 6 D were entered in the "medium myopia" group, and patients with attempted correction exceeding 6 D were classified in the "high myopia" group.

Preoperative assessment consisted of a complete ophthalmic examination, featuring slit lamp biomicroscopy, uncorrected and best spectacle-corrected visual acuity measurements, cycloplegic refraction using an autorefractometer, applanation tonometry, altimetric corneal topography with pupillometry (Orbscan, Orbtek, Salt Lake City, Utah).

Peripapillary RNFL thickness was assessed using the NFA type II GDx.

*GDx technical features:* The instrument is a scanning laser ophthalmoscope with a polarisation modulator, a cornea polarisation compensator, and a detection unit. The light source a near-infrared diode laser (wavelength 780 nm) in which the state of polarisation is changed by passing the linearly polarised laser beam through a constantly rotating quarter-wave retarder. The parallel microtubules of the nerve fibers form a birefringent medium and change the polarisation of the light reflected and/or backscattered from the eye to the detector (6) After emerging from the eye, the refracted light follows the same path until it is separated from the illuminating light beam by a non-polarising beam splitter and the polarisation of the light is then analysed by the detection unit.

Fourier analysis is done on the data to calculate the retardation at the measuring location. Anterior segment birefringence is automatically compensated, so the resulting change in the state of polarisation is proportional to the thickness of the RNFL. A scan unit deflects the illuminating laser beam to an adjacent retinal position and the whole procedure is repeated. A complete scan consists of 256 x 256 individual retinal positions (pixels) (4,6). Immediately after acquiring the data, a computer algorithm computes the retardation at each retinal position and a retardation map is created. The optic disk margin was approximated by a circle placed around the inner margin of the peripapillary scleral ring by the operator, and retardation data was automatically obtained for three circles concentric with the disk margin (1.5, 1.8, 2.3 disk diameters). Mean retardation along each circle was recorded in 16 equal sectors at 22.5 degree intervals, forming the four quadrants of the retinal image. With 0° to 22.5° considered as temporal and 157.5° to 180° as nasal, the superior quadrant was defined as between 45° and 135° and the inferior region between 225° and 315° (4). An external fixating light was used and the field size was set to 15 x 15°. The pupils were undilated. The optic nerve head was placed in the centre of the display and at least three good quality images were obtained. The *mean image* was calculated from the corresponding three images using the software supplied with the instrument. The disk margin was defined separately on each baseline image. Thickness was measured along a line at 1.5 disc diameters concentric with the disk margin, applying default 90° quadrant positions. The reliability of the measurements was assessed by calculating the mean thickness deviation. If it was more than 5μ, the measures were not reliable.

*Surgical technique:* All surgical procedures were performed by the same surgeon (M.V.), using a Laser-scan 2000 (Lasersight, Orlando, FL), which reprofiles the corneal surface by means of a galvanometric scanning delivery system (flying spot). Its technical features were: repetition rate 100 Hz, fluence 160 mJ/cm$^2$, beam diameter 1 mm. The laser ablation algorithm enabled the operator to perform corneal ablations using a single pass multizone technique; the number of zones and their smallest and largest diameters were computed by integrating preoperative mesopic pupillometric measurements with the depth of the corneal ablation zone. Further safety devices were the active eye-tracker, which automatically centered the ablation over the pupil and the internal power stabilizator, which ensured uniform energy delivery throughout the treatment (12,13).

Our surgical procedure provided a single-zone PRK with a wide ablation profile (up to 7 mm) for low myopia (less than -3.00 D), whereas for moderate and severe myopia two to four zones were used, with a slightly narrower profile (from 5 to 6.7 mm), in order to obtain ablation zones no deeper than 100 µm. Topical anaesthetic drops were instilled (oxybuprocaine hydrochloride 0.4%, Novesina, Sandoz, Italy), the epithelium within the ablation zone was removed with a blunt Desmarres blade and then the laser ablation was performed. On completion of the surgical treatment, all patients received one drop of fluorometholone 0.1% acetate (Flarex, Alcon, Milano, Italy), ofloxacin (Exocin, Allergan, Roma, Italy) and flurbiprofen (Ocufen 40, Allergan, Roma, Italy). A soft contact lens (Acuvue, Johnson and Johnson, Jacksonville, FL) was applied. All patients were advised to take additional analgesic tablets (ketorolac tromethamine, Lixidol, 10 mg tablets, Farmitalia, Milano, Italy) if pain was not controlled properly by topical therapy. The first postoperative visit was scheduled after complete reepithelialization (3-5 days).

After this first postoperative phase, the contact lenses were removed and patients received a topical steroid: fluorometholone 0.1% acetate (Flarex, Alcon, Milano, Italy) was prescribed for myopia up to -5 D, and dexamethasone (Luxazone, Allergan, Roma, Italy) was administered in cases with myopia exceeding -5 D. Drugs were dispensed four times a day for one month and then in decreasing frequency every three weeks.

*Postoperative patient selection:* A general examination was done at each time point. Corneal haze was assessed according to the international grading scale (14). Postoperative exclusion criteria were corneal haze more than 1, IOP higher than 24 mmHg after steroid therapy, changes in lens transparency. At the 5-day, 3-month and 6-month time points, RNFL was measured again with the Gdx NFA.

*Statistics:* All data were recorded in a spreadsheet and analysed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA, software version 5.0) and Winks Kwikstat (TexaSoft, Cedar Hill, TX, USA, software version 4.5) as statistical analysis tools ($p<0.05$). Preoperative measurements were compared with postoperative ones (six months) for each subgroup of myopic correction. Mean postoperative-preoperative differences for each subgroup were compared with a one-way ANOVA. If a difference was significant, ANOVA was repeated with Bonferroni's correction for multiple comparison ($p<0.016$). Mean RNFL thickness at baseline and at the 5-day, 3-month and 6 month time points were compared with Student's t-test for repeated measures. The postoperative change in RNFL thickness was correlated with ablation depth by simple linear regression analysis.

This study had a power of 80% to detect a difference of 0.001 microns in mean RNFL thickness given a sample size of 60 subjects with 20 in each treatment group. In order to increase the power of the study, we doubled this number and enrolled 120 patients (15).

## RESULTS

Applying the inclusion and exclusion criteria, we excluded 30 patients from the study. Twenty-three did not meet the inclusion criteria and eight were excluded after PRK, since four had developed corneal haze more than 1 and four were steroid "responders". Thus, 120 patients (120 eyes) were enrolled in the study, with 40 patients for each group of attempted myopic correction. Preoperative characteristics are shown in Table I. Comparing the mean differences for each subgroup, significant differences were found in four GDx parameters: symmetry, superior maxima and average thickness had decreased significantly six months after PRK, while the inferior maxima increased (Tab. II).

Repeating the ANOVA test with Bonferroni's correction for multiple comparisons, significant P values were detected for the symmetry (the high myopia group was significantly greater than the low an

**TABLE I -** PATIENTS' MAIN CHARACTERISTICS (N=120 EYES)

| **Preoperative independent variables** | **Mean ± SD** |
|---|---|
| Age (years) | 29.8 ± 8.23 |
| Sex (M/F) | 68/52 |
| Degree of myopia (D) | 6.54 ± 2.16 |
| Degree of astigmatism (D) | 1.15 ± 0.22 |
| Pachymetry (μm) | 506.89 ± 58.20 |
| Intraocular pressure (mmHg) | 14.84 ± 2.12 |

medium myopia groups), and superior maxima (significant difference only between high and medium myopia groups). Average thickness was significantly more reduced after PRK in the high myopia group than in the other groups (Tab. II). Although the difference in the change in inferior maximum was significant by one-way ANOVA, there was no significant difference in any comparison using Bonferroni's correction, indicating that the significant ANOVA p value for this parameter occurred by chance because of the number of tests.

Mean RNFL thickness did not change with time ($p = 0.884$) as shown in Figure 1.

Ablation depth was correlated with a change in RNFL thickness at the sixth postoperative month. The

**TABLE II -** CHANGES IN GDx PARAMETERS FROM BASELINE TO SIX MONTHS AFTER PRK IN RELATION TO MYOPIA (MEAN ± SD)

| **GDx NFA II Parameter** | **Low myopia (N=40)** | **Medium myopia (N=40)** | **High myopia (N=40)** | **ANOVA p-value** | **Bonferroni's correction *** |
|---|---|---|---|---|---|
| Symmetry | -0.190 ± 0.020 | -0.210 ± 0.050 | -0.240 ± 0.110 | 0.008 | High-Low |
| Superior ratio | -0.367 ± 0.233 | -0.458 ± 0.598 | -0.532 ± 0.573 | 0.334 | – |
| Inferior ratio | -0.196 ± 0.465 | -0.217 ± 0.486 | -0.319 ± 0.389 | 0.426 | – |
| Superior/nasal | -0.562 ± 0.231 | -0.548 ± 0.252 | -0.510 ± 0.243 | 0.612 | – |
| Max modulation | -0.489 ± 0.218 | -0.467 ± 0.207 | -0.525 ± 0.189 | 0.445 | – |
| Superior maximum | -1.583 ± 2.43 | -1.391 ± 2.12 | -2.579 ± 1.65 | 0.027 | High-Medium |
| Inferior maximum | 5.03 ± 2.73 | 4.92 ± 2.89 | 6.35 ± 2.65 | 0.039 | – |
| Number | 16.22 ± 13.47 | 15.85 ± 10.34 | 18.23 ± 13.89 | 0.665 | – |
| Ellipse modulation | -0.634 ± 1.41 | -0.827 ± 1.35 | -0.911 ± 1.55 | 0.678 | – |
| Average thickness | -4.6 ± 20.88 | -4.05 ± 23.49 | 9.15 ± 26.05 | 0.015 | High-Medium, High-Low |
| Ellipse average | 5.62 ± 17.89 | 6.93 ± 19.72 | 6.57 ± 12.56 | 0.939 | – |
| Superior average | 1.45 ± 2.64 | 2.35 ± 5.92 | 1.28 ± 6.23 | 0.614 | – |
| Inferior average | 5.45 ± 3.67 | 8.53 ± 6.23 | 6.78 ± 7.45 | 0.074 | – |
| Superior Integral | 0.024 ± 0.044 | 0.022 ± 0.039 | 0.021 ± 0.043 | 0.949 | – |

* Bonferroni's correction for multiple comparisons was applied after ANOVA. This column shows the pairs whose p value is significant ($p<0.016$)

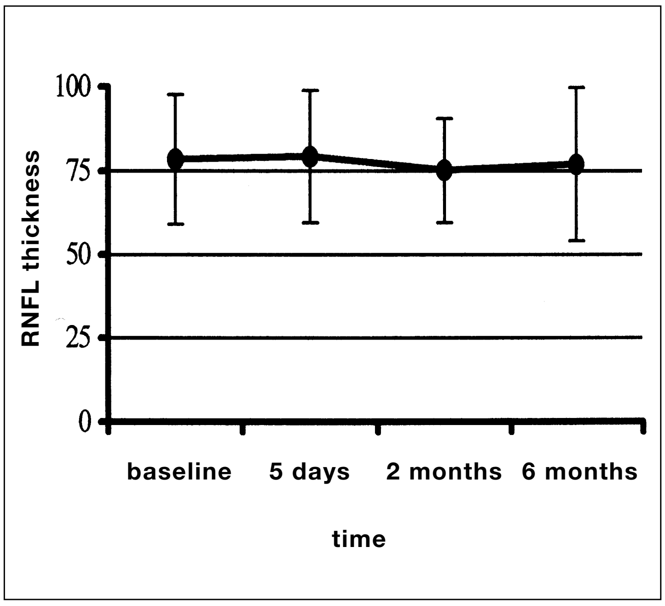


**Fig. 1** - *Change in RNFL thickness with time.*

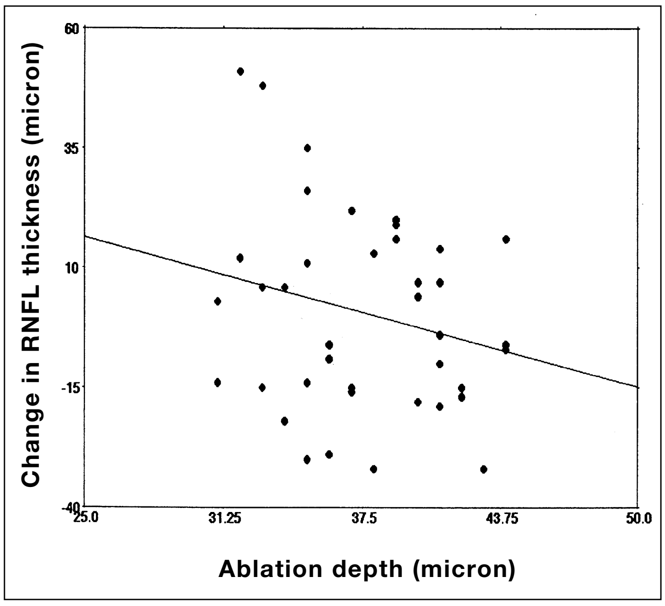


**Fig. 2** - *Relationship between ablation depth and change in RNFL thickness six months after PRK in the low myopia group.*

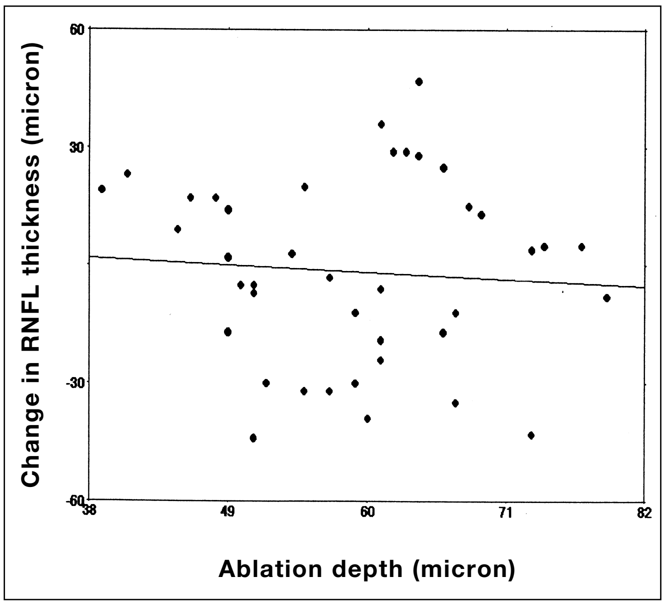


**Fig. 3** - *Relationship between ablation depth and change in RNFL thickness six months after PRK in the medium myopia group.*

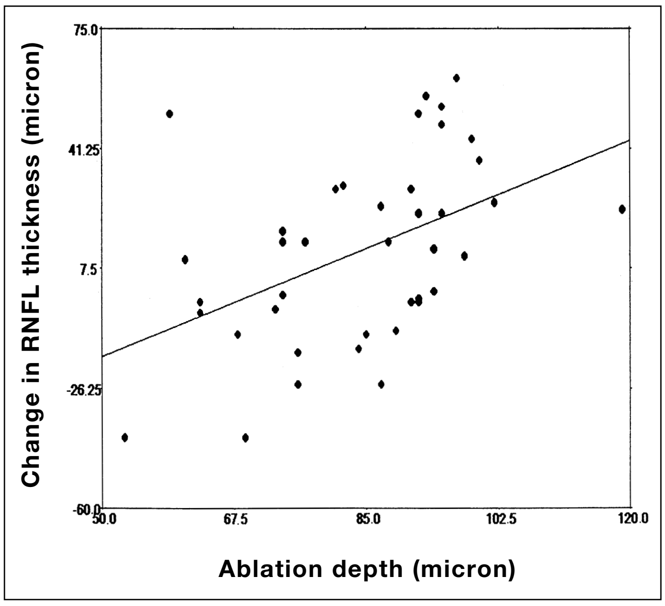


**Fig. 4** - *Relationship between ablation depth and change in RNFL thickness six months after PRK in the high myopia group.*

lack of association betwen these variables in low myopia and medium myopia groups confirms that ablation depth does not affect the results (p = 0.158 and p = 0.602, respectively), (Figs. 2 and 3). On the other hand, a significant relationship between ablation depth and change in RNFL thickness was established in the high myopia group, yielding a slope of 0.246 ($R^2 = 0.214$, p = 0.003) as shown in Figure 4.

## DISCUSSION

Scanning laser polarimetry was found to be a quick and simple technique for RNFL analysis and provides reproducible measurements, useful for detecting RNFL changes and monitoring glaucomatous progression (5, 16). The instrument does not measure RNFL thickness directly but relies on the retardation of polarised laser light, which is proportional to total ocular birefringence. As reported by Dreher and Reiter, these retardation measurements also reflect the combined effects of cornea and lens (6). This additional polarisation is neutralised by a compensatory unit, although the compensation range can be exceeded by high polarising surfaces (peripheral cornea, chorioretinal scars, and artefacts) (5).

The GDx Nerve Fiber Analyser II can assess 14 different parameters. Some, such as symmetry or superior and inferior maxima, were found to be changed, but we considered they were not really important for the aim of the study. “Symmetry” in this case assesses the changes in symmetry between different retinal quadrants, but acquisition bias could also affect this parameter. Some other parameters, however, like average thickness, superior and inferior maxima, appeared to be more useful in differentiating normal subjects and glaucoma suspects (6, 17). If superior maxima and other parameters are most likely correlated to RNFL thickness changes,average thickness was not based on topographical evaluations of the retinal bundles and could possibly be affected more by changes in corneal birefringence than other parameters. We therefore focused our attention on this parameter, evaluating its changes with time.

We repeated the measurements five days after PRK, when reepithelialization and the inflammatory reaction occur, checking for changes in cellularity and hydration (18, 19). RNFL thickness did increase in our study, although not significantly.

Other important time points are three months after PRK, when postoperative therapy is completed and complications are easily detectable and six months, when stromal remodelling is generally completed and many factors which may cause optical aberrations (e.g. corneal haze) subside (7, 20).

Previous investigations reported no significant changes after PRK in any GDx parameter (10) but our findings agree only for myopic corrections lower than 6 D. This could be explained by the mean attempted correction in both samples. Choplin et al included only patients with corrections of 3.4 ± 1.9 D, whereas we planned greater myopic correction, in order to avoid data limited to just one class of patients. No significant difference was seen in the low and medium myopic groups, but postoperative RNFL thickness was significantly lower than baseline in the high myopia group.

This might be related either to a deeper ablation or to a wider treated area. We therefore investigated whether a relationship could be established between ablation depth and postoperative RNFL thickness (six months). The relationship was significant only in the high myopia group. This confirms the theoretical assumption that deeper ablations, to correct higher myopia, may cause greater changes in corneal birefringence. This could be related either to the flattening of the central cornea and to its oblated profile or to the effect of the transition zone (where the ablation area joins the normal cornea) on corneal birefringence, known as the “edge effect” (21, 22).

Many variables, however, can influence corneal birefringence postoperatively, especially in cases of high myopia. This might have contributed to the wider range of postoperative RNFL measurements, expressed by a larger SD at six months (8, 24). On the other hand, high axial myopia may be a factor affecting image acquisition during scanning laser polarimetry, affecting RNFL accuracy (25, 26).

Though RNFL measurements in the high myopic group must be evaluated carefully, there was clearly a significant change in RNFL. Despite refined laser engineering and all the safety devices used to ensure a smooth ablated surface, the homogeneity of the energy delivery and the reduction of steep ablation margins by using a multizone technique, there are always some patients who present an abnormal corneal healing response (8, 13, 21, 23). This could lead to visual impairment or subjective problems, but some cases may remain symptom-free with only corneal birefringence decreasing.

As RNFL measurements decrease in glaucoma or after PRK for high myopia, this change must be evaluated carefully when populations at risk of glaucoma are screened. The ablation area corresponds to the zone penetrated by the polarised light and it is therefore very easy to misinterpret morphological and functional changes, giving false positive results. Therefore we suggest modifying the mathematical model described by Dreher and Reiter, adjusting the assumed corneal polarisation properties when performing scanning laser polarimetry on these patients, in order to compensate corneal birefringence entirely (6). As the GDx NFA type II is currently one of the most useful tools for screening and detection of early glaucoma, the relationship between RNFL thickness and ablation depth merits further investigation.

RNFL scans must always be interpreted carefully as abnormal scans in patients who have undergone PRK may be due to birefringence changes after laser ablation and may not be indicative of RNFL damage. RNFL scans in highly myopic patients must always be analysed in relation to the preoperative myopia and it may be more appropriate to use the scan as a baseline for subsequent assessments.

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