

EXTENDED REPORT

Corneal birefringence changes after laser assisted in situ keratomileusis and their influence on retinal nerve fibre layer thickness measurement by means of scanning laser polarimetry

M Centofanti, F Oddone, M Parravano, L Gualdi, M G Bucci, G Manni

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Aim: To evaluate changes in corneal polarisation properties and their influence on peripapillary retinal nerve fibre layer (RNFL) thickness measurements after laser assisted in situ keratomileusis (LASIK) by means of scanning laser polarimetry (SLP) with variable corneal polarisation compensator (VCC) in normal white subjects.

Methods: SLP was performed by means of GDx VCC on 32 eyes of 32 normal subjects who underwent LASIK for ametropia correction. Corneal polarisation axis and magnitude and RNFL thickness were measured before and 8 days after LASIK. RNFL thickness data and corneal polarimetric data of one randomly selected eye per subject were analysed by the Wilcoxon signed ranks test. Correlations between corneal ablation depth, corneal polarimetric changes, and RNFL thickness changes were investigated using Spearman's rho test.

Results: The corneal polarisation axis significantly shifted from 15.1° (17.0°) to 6.9° (12.9°) ($p=0.00006$) after LASIK and this change showed a strong correlation with corneal ablation depth ($\rho=-0.7$, $p=0.00002$). Among GDx parameters, TSNIT, SUP, and SD showed significant changes after LASIK and for SUP and SD these changes were well correlated with the shift in corneal polarisation axis ($\rho=0.54$, $p=0.03$ and $\rho=0.45$, $p=0.01$, respectively). SUP and SD changes were neutralised after compensating for corneal polarimetric changes but not TSNIT changes. NFI, a discriminating parameter, was found to be affected after LASIK only after compensating for corneal polarimetric changes.

Conclusions: LASIK induces a shift in corneal polarisation axis which is responsible for inaccuracies in RNFL thickness measurements. A customised compensation for corneal polarimetric changes after LASIK allows normalisation of some of the thickness parameters except for TSNIT and NFI.

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Scanning laser polarimetry (SLP) is a technology that allows a reproducible and quantitative evaluation of the peripapillary retinal nerve fibre layer thickness (RNFL).^{1–4} It is based on the principle that a polarised scanning laser beam changes its polarisation status when passing through a form birefringent tissue—that is, a tissue organised in parallel structures with a diameter smaller than the wavelength of the illuminating beam. The polarisation status change, also known as retardation, may be quantified by detecting the phase shift between the illuminating polarised laser beam and the light backscattered from the eye.

The retinal nerve fibre layer is mainly made up of unmyelinated ganglion cell axons containing microtubules organised in parallel bundles. This organisation is responsible for the form birefringence properties of the tissue.⁵ Studies in animal models have demonstrated that the amount of retardation of the backscattered light coming from the eye is linearly related to the thickness of RNFL.⁶ Nevertheless, the RNFL is not the only birefringent tissue of the eye. The cornea and the lens also have birefringent properties^{7–8} that contribute to the total retardation so that the accuracy of RNFL measurements is dependent on the ability to quantify and neutralise anterior segment birefringence (polarisation axis and its magnitude).⁹ A linear relation between corneal polarisation axis and posterior segment retardation has been documented.¹⁰ To compensate anterior segment birefringence a variable corneal polarisation compensator (VCC) has been recently integrated in the commercially available scanning

laser polarimeter (GDx) allowing compensation for individual corneal birefringence.

Several authors observed that the accuracy of RNFL thickness measurement as measured by GDx is affected by uncomplicated laser assisted in situ keratomileusis (LASIK),^{11–14} a refractive surgery technique that combines lamellar corneal surgery with excimer laser stromal ablation. Nevertheless, little is known about how corneal birefringence behaves following corneal stromal thinning. The aim of this study was to evaluate changes in corneal polarisation properties and their influence on RNFL thickness measurements after LASIK, by means of GDx VCC.

METHODS

Thirty two eyes of 32 healthy subjects undergoing LASIK for ametropia were enrolled in the study. One eye was randomly chosen if both were scheduled for LASIK and eligible for the study. The study protocol was approved by the ethics committee of the university and each subject signed a written informed consent before enrolment.

Inclusion criteria were 18 years of age or older, a spherical refractive error within -10 dioptres and $+3$ dioptres, astigmatism within plus or minus 3 dioptres, absence of

Abbreviations: LASIK, laser assisted in situ keratomileusis; RNFL, retinal nerve fibre layer; SLP, scanning laser polarimetry; VCC, variable corneal compensator

ophthalmic disease, or abnormal conditions other than ametropia.

Exclusion criteria were best corrected visual acuity worse than 20/40, the presence of peripapillary atrophy, intraocular pressure more than 22 mm Hg, and any previous ocular laser or surgery.

Each subject underwent a complete ophthalmological examination before LASIK, including uncorrected and best corrected visual acuity, slit lamp biomicroscopy, Goldmann applanation tonometry, and dilated indirect ophthalmoscopy using a slit lamp and a hand held 90 dioptre lens. Moreover, automated refractometry before and after cycloplegia, corneal topography, and pachymetry (SP-9000 Konan Noncon Robo Pachy) was performed before LASIK. Subjects were asked not to wear contact lenses for at least 4 weeks before the visit.

SLP with GDx VCC was performed to assess and quantify the corneal polarisation axis and magnitude and to measure peripapillary RNFL thickness.

The GDx measurement technique has already been described in detail elsewhere.^{15–23} Briefly, a near infrared laser beam (780 nm) is scanned in a raster pattern on the retinal surface.²⁴ The raster scan captures an image with a field of 40° horizontally by 20° vertically, including both the peripapillary and the macular region.^{25–26} For each measurement, the GDx VCC generates two images: a reflectance image and a retardation image. The reflectance image is generated from the light reflected directly back from the surface of the retina. The retardation image is the map of retardation values and is converted into RNFL thickness based on a conversion factor of 0.67 nm/μm.²⁷

Anterior segment birefringence is quantified and neutralised using the VCC integrated in the GDx. The specific axis and magnitude of the anterior segment birefringence is determined by first imaging the eye without compensation. The macular region of this image is then analysed to determine the axis and magnitude of the anterior segment birefringence.²⁶ The macular region birefringence is physiologically uniform and symmetric as a result of the radial distribution of Henle's fibre layer. However, in uncompensated scans, a non-uniform retardation pattern is present in the macula because of the birefringence from the anterior segment. The axis and magnitude values from the anterior segment can be computed by analysing the non-uniform retardation profile around the macula.²² The axis of the anterior segment birefringence is determined by the orientation of the "bow tie" birefringent pattern in the macula and the magnitude of the anterior segment birefringence is calculated by analysing the circular profile of the birefringence in the macula according to equations described in detail by Zhou and Weinreb.²⁶

In this study, SLP with GDx VCC (software version 5.3.1) was performed without pupil dilatation before LASIK (visit 1) and 8 days after LASIK (visit 2). A macular scan was obtained to individually quantify corneal birefringence at

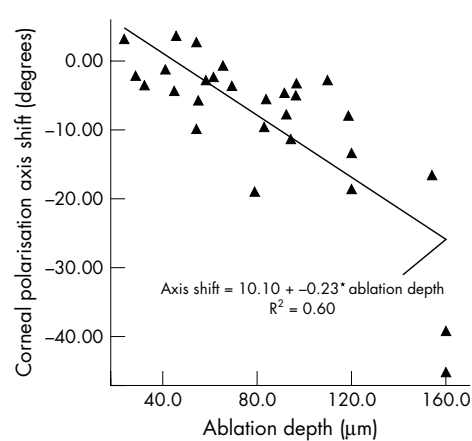


Figure 1 Linear correlation analysis between corneal ablation depth and corneal polarisation axis shift.

both visits 1 and 2. Moreover, three RNFL scans were obtained: (1) at visit 1, after anterior segment birefringence compensation, (2) at visit 2, without re-compensation for anterior segment birefringence (using the pre-LASIK anterior segment compensation values), (3) at visit 2, with a new anterior segment birefringence compensation. The optic disc reference ring was automatically superimposed by the software at the optic disc edge on the reflectance image. If the automatic alignment was judged to be poor, the operator was allowed, for the first examination, to adjust both size and position. The same size and position were then automatically carried forward for the following examinations. The reference ring on the macular scans was automatically superimposed and centred at the fovea by the software without any intervention by the operator. Atypical scans were rejected and were not used for study purposes.

Four RNFL thickness parameters (TSNIT, SUP, INF, SD), one discriminating parameter (NFI), corneal polarisation axis, and magnitude were considered for the analysis and are described in detail in table 1.

Excimer laser stromal ablation was performed for all eyes with Ladarvision 4000 using an optical zone of 6.5 mm, a flying spot of 0.8, and the Ladar tracker closed loop of 4000 Hz. The corneal flap was created with a superior hinge for all eyes using the Hansatome down-up Chiron Bauch & Lomb microkeratome with a suction ring of 9.5 mm. Flap thickness was set at 160 μm.

Statistical analysis

The results were summarised by calculating the mean and standard deviation. The Wilcoxon signed ranks method was used to test for differences between paired scores before and after LASIK. Spearman's rho was used to test correlations between corneal ablation depth, corneal birefringence changes, and changes in RNFL thickness parameters after LASIK.

RESULTS

All the 32 enrolled subjects underwent LASIK for ametropia correction and completed the study without adverse events, but only 29 (mean age 37.5 (SD 12.0) years, 13 women and 16 men) were considered for the statistical analysis, because three eyes showed atypical GDx scans before LASIK and were excluded. Mean preoperative spherical equivalent was -4.1 (3.9) D, mean central corneal thickness was 558.8 (30.4) μm, and mean corneal ablation depth was 80.8 (38.1) μm. Among corneal birefringence parameters, mean polarisation magnitude did not show statistically significant changes following corneal ablation, while mean corneal polarisation axis

Table 1 Definition of the GDx parameters analysed

TSNIT	the average RNFL thickness around the calculation circle
average	
SUP	the average RNFL thickness in the superior 120° region of the calculation circle
INF	the average RNFL thickness in the inferior 120° region of the calculation circle
SD	this measure represents the standard deviation of the values contained in the calculation circle
NFI	it is a global measure based on the entire RNFL thickness map calculating using an advanced form of neural network to discriminate normal from glaucoma

Table 2 Refractive changes and corneal polarimetric changes after uncomplicated LASIK

	Pre-LASIK	Post-LASIK	p Value
Spherical equivalent (D)	-4.1 (3.9)	-0.2 (0.6)	0.00008
Corneal polarisation magnitude (nm)	40.0 (13.9)	41.5 (12.7)	0.19
Corneal polarisation axis (degrees)	15.1 (17.0)	6.9 (12.9)	0.00006

significantly shifted from 15.1° (17.0°) to 6.9° (12.9°) ($p = 0.00006$) (table 2). A strong correlation was found between corneal ablation depth and corneal polarisation axis shift ($\rho = -0.7$, $p = 0.00002$) (fig 1).

Of the GDx parameters, TSNIT, SUP, and SD showed a statistically significant change after LASIK when measured without post-LASIK corneal birefringence re-compensation. The new corneal birefringence compensation after LASIK allowed the neutralisation of SUP and SD changes but not TSNIT changes. INF did not change after LASIK either with or without the new corneal compensation. NFI was found to be altered after LASIK only after the new compensation (table 3).

The SUP and SD changes observed after LASIK when measured without a post-LASIK corneal compensation were found to correlate with LASIK induced corneal polarisation axis shift ($\rho = 0.54$, $p = 0.03$ and $\rho = 0.45$, $p = 0.01$ respectively) as shown in table 4.

DISCUSSION

In this study, LASIK corneal stromal thinning was found to significantly influence corneal polarisation properties as measured by GDx VCC and was specifically found to induce a shift in corneal polarisation axis, which is responsible for inaccuracies in RNFL thickness measurement. Moreover, the shift in corneal polarisation axis was observed to be strongly related to the corneal stromal ablation depth.

Several authors reported changes in RNFL thickness measurements following uncomplicated LASIK procedure, which was believed, but to our knowledge never demonstrated, to be caused by corneal alterations induced by the refractive treatment.^{11–14}

The results of this investigation showed a strong relation between corneal stromal ablation depth and the shift in corneal polarisation axis, confirming that LASIK stromal thinning is responsible for significant changes in corneal birefringence.

The influence of corneal polarisation axis on RNFL thickness measurements by SLP has been previously investigated and a linear relation was found between the corneal polarisation axis orientation and posterior segment retardation values.¹⁰ Moreover, a lack of correction of the corneal polarisation axis was found to significantly influence the discriminating power of SLP between normal eyes and eyes with glaucoma.²⁸

In the present study we also investigated whether corneal birefringence changes were related to changes in RNFL

thickness measurement after LASIK, by measuring corneal birefringence before and after LASIK and correlating the LASIK induced corneal birefringence changes with the LASIK induced RNFL thickness changes when measured without performing a new corneal polarisation compensation. In this way, it was possible to observe that several GDx parameters were significantly modified after LASIK when measured without compensating the corneal birefringence changes induced by the corneal thinning. Specifically, the average RNFL thickness around the calculation circle (TSNIT), the average RNFL thickness in the superior 120° of the calculation circle (SUP), and the standard deviation of the values contained in the calculation circle (SD) were found to be significantly altered after LASIK but only SUP and SD showed a relation to the corneal polarisation axis shift. Moreover, SUP and SD were the only parameters that showed normalisation after the corneal polarisation axis shift was compensated by means of a new corneal compensation. The lack of correlation between changes in corneal birefringence and TSNIT changes after LASIK might be explained when considering that this parameter represents the average thickness around the entire 360° of the calculation circle, thus also including the average thickness of the inferior 120° (INF), which was not affected by changes in corneal birefringence induced by LASIK. Moreover, TSNIT changes after LASIK also persisted after the new corneal compensation. This could be because changes in corneal birefringence might affect the temporal and nasal areas which are included in the TSNIT parameter and are not so far separately analysable.

Similar results were reported in a previously published study by Holló *et al*,¹¹ where the SUP and INF were found to be the most stable parameters after customised corneal compensation while SUP to temporal ratios and INF to temporal ratios were found to be decreased compared to the pre-LASIK values, suggesting that customised compensation, when used after LASIK, might not be optimal in the temporal quadrant. The explanation for this finding is only speculative and it might be that the highly reflective temporal sector is compensated with difficulty by the machine algorithm because of an irregular and atypical birefringence pattern created after LASIK, thus influencing global and ratio parameters after LASIK.

Moreover, it should be considered that the superior position of the corneal flap hinge might have influenced the retardation changes observed in the SUP sector after LASIK. As previously reported by Holló *et al*²⁹ the thickness

Table 3 RNFL thickness values before and after uncomplicated LASIK

	Pre-LASIK (VCC1)	Post-LASIK (VCC1)	Mean change from pre-LASIK	p Value	Post-LASIK (VCC2)	Mean change from pre-LASIK	p Value
TSNIT	54.5 (5.4)	57.0 (6.0)	2.5 (3.7)	0.01*	56.0 (4.7)	1.6 (2.8)	0.006*
SUP	64.9 (8.8)	62.9 (8.4)	-2.1 (3.8)	0.009*	65.9 (8.0)	1.0 (2.8)	0.07
INF	64.7 (7.1)	65.8 (8.7)	1.1 (6.3)	0.3	65.2 (8.0)	0.4 (4.1)	0.9
SD	22.1 (4.4)	19.3 (5.1)	-2.8 (4.2)	0.0005*	21.5 (3.6)	-0.6 (2.5)	0.08
NFI	21.7 (8.8)	23.1 (8.7)	1.4 (4.1)	0.06	20.0 (6.8)	-1.7 (4.0)	0.03*

p Values are referred to the mean difference between post-LASIK and pre-LASIK values (Wilcoxon signed ranks test).

Table 4 Correlation between corneal polarisation axis shift and RNFL thickness changes after LASIK without (1) and with (2) a post-LASIK corneal polarisation compensation (Spearman's rho test)

GDx parameters	Spearman's rho (1)	p Value	Spearman's rho (2)	p Value
TSNIT	-0.24	0.3	-0.10	0.6
SUP	0.54	0.003*	-0.28	0.1
INF	0.18	0.3	0.06	0.8
SD	0.45	0.01*	-0.05	0.8
NFI	-0.26	0.2	0.2	0.2

parameters provided by the GDx can be divided into two groups: the parameters representing the centre of the polarimetric image and the parameters calculated from all pixels of the total image area, including information from the peripheral parts of the image. The significance of the distinction between the two kinds of parameters is that the central part of the polarimetric image is imaged via the laser treated central corneal area, while the information from the more peripheral parts of the retina might also be influenced by additional factors originating from the paracentral cornea, which represents a transition between the treated and untreated areas where the hinge is placed. It should thus be considered that the paracentral area of the cornea where the hinge is placed (approximately 4.5 mm from the center of the cornea) is unlikely to influence the thickness parameters investigated in this study calculated from the laser treated central corneal area (TSNIT, SUP, INF, SD).

The NFI response after LASIK was also studied, and showed paradoxical behaviour; it remained stable when calculated without compensating for changes in corneal birefringence, while it changed significantly when the new corneal birefringence was compensated. NFI is a global measure based on the entire RNFL thickness map calculated using an advanced form of neural network trained to find differences between glaucomatous and non-glaucomatous eyes, and therefore the LASIK induced atypical polarisation pattern might be neutral for NFI. It was reported to be the best parameter to discriminate normal from glaucomatous eyes,³⁰ although, considering the results of this study, its discriminating power has to be considered with caution in eyes that underwent refractive surgery.

Finally, considering what was previously reported by Holló *et al*,²⁹ that the LASIK induced changes of thickness parameters are maximal in the early postoperative period and tend to change with time, it would be interesting to investigate the corneal birefringence behaviour in relation with thickness parameter behaviour in the late post-LASIK period of life.

In conclusion, this study provides information on how corneal polarimetric properties behave after corneal stromal thinning obtained by LASIK, and shows how the LASIK induced corneal polarisation axis shift affects peripapillary RNFL measurements. Moreover, it shows that a customised compensation for corneal polarimetric changes after LASIK allows the normalisation of several thickness parameters, with the exception of average RNFL thickness and the discriminating parameter NFI, which should be critically considered during the follow up of patients who underwent LASIK for ametropia correction.

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