Bilateral morphometric analysis of corneal sub-basal nerve plexus in patients undergoing unilateral cataract surgery: a preliminary in vivo confocal microscopy study

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ABSTRACT

Aims To evaluate bilateral morphometric changes of corneal sub-basal nerve plexus (CSNP) occurring after unilateral cataract surgery by in vivo confocal microscopy (IVCM) images analysed with automated software.

Methods IVCM was performed before (V0) and 1 month after surgery (V1) in both operated eyes (OEs) and unoperated eyes (UEs) of 30 patients. Thirty age and sex-matched subjects acted as controls. Corneal nerve fibre density (CNFD), corneal nerve branch density (CNBD), corneal nerve fibre length (CNFL), corneal nerve total branch density (CTBD), corneal nerve fibre area (CNFA), corneal nerve fibre width, corneal nerve fractal dimension (CNFrD) and dendritic cells density were calculated.

Results Mean CNFD, CNBD, CNFL, CTBD, CNFA and CNFrD significantly decreased at V1 versus V0 in both eyes (respectively, 15.35±7.00 vs 21.21±6.56 n/mm^2 in OEs and 20.11±6.69 vs 23.20±7.26 in UEs; 13.57±12.16 vs 26.79±16.91 n/mm^2 in OEs and 24.28±14.88 vs 29.76±15.25 in UEs; 9.67±3.44 mm/mm^2 vs 13.49±3.42 in OEs and 12.53±3.60 vs 14.02±3.82 in UEs; 22.81±18.77 vs 42.25±24.64 n/mm^2 in OEs and 38.06±20.52 vs 43.93±22.27 in UEs; 0.0040±0.0021 vs 0.0058±0.0020 mm^2/mm^2 in OEs and 0.0049±0.0016 vs 0.0057±0.0019 in UEs; 1.418±0.058 vs 1.470±0.037 in OEs and 1.466±0.040 vs 1.477±0.036 in UEs; always p<0.049).

Conclusion Patients undergoing cataract surgery exhibit bilateral alterations of CSNP. This finding could have broad implications in the setting of sequential cataract surgery.

INTRODUCTION

Modern cataract surgery has reached high standards of safety and visual performance. However, patient's satisfaction after surgery is not always consistent with these advances. The main reason is related to ocular discomfort symptoms that patients frequently report in the operated eye, particularly in the early postoperative period. Although the pathophysiological mechanisms underlying cataract surgery-induced dry eye are multifactorial, the full-thickness nerves transection at the site of the wound represents a chore mechanism of this type of iatrogenic dry eye.[1 2] Previous in vivo confocal microscopy (IVCM) studies showed that the injured nerves degenerate after surgery resulting in reduced sub-basal nerve plexus (SNP) density and reflectivity and increased beading in the operated eye.[3 4]

Recently, the contralateral subclinical impairment of SNP has been detected by means of IVCM in unilateral herpetic keratitis thanks to incidental control observations obtained by Hamrah and collaborators in eyes that did not present any abnormality when examined by conventional slit-lamp biomicroscopy.[5] Thereafter, the increasing evidence provided from the same research group pointed out the presence of bilateral coordinated interaction between the nervous and the immune systems of affected and unaffected eyes in other ocular diseases, including neurotrophic and microbial keratitis.[6–8] Additionally, unilateral 360° full-thickness cornea incision during keratoplasty has been demonstrated to induce a sympathetic response that permanently abolishes immune privilege of subsequent corneal allografts, even in the opposite eye.[9]

Taking into account the nervous interdependence between both eyes, we hypothesise that microincisional cataract surgery may determine contralateral corneal nerves changes as well. Therefore, the purpose of this work was to evaluate the morphometric analysis of corneal SNP in both eyes of patients undergoing unilateral cataract surgery using a fully automated software for the analysis of IVCM images ('ACCMetrics'). To the best of our knowledge, this is the first IVCM study describing the sympathetic effect of unilateral cataract surgery on contralateral corneal nerves.

MATERIAL AND METHODS

Subjects

This prospective study conducted between April 2019 and February 2020 enrolled volunteers scheduled to undergo phacoemulsification and intraocular lens implantation at the University Eye Clinic of Genoa, Policlinico San Martino, Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, Genoa, Italy. Healthy sex-matched and age-matched subjects acted as controls. Inclusion criteria were visually significant senile cataract (NC2–NC5 regarding Lens Opacities Classification System III)[10] in at least

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one eye, corrected distance visual acuity decreased by minimum to 0.1 logMAR in the affected eye, and willingness to volunteer for the study after giving written informed consent. The exclusion criteria for both groups included corneal and ocular surface diseases (including dry eye), glaucoma, pseudoexfoliation syndrome, zonular weakness, history of ocular surgery, active or past inflammations, reduced compliance, age younger than 50 years, any usage of eye drops or participation in another clinical study within 1 month prior to study initiation. In addition, patients suffering from other predisposing conditions for peripheral or central neuropathy, in particular diabetes, multiple sclerosis, ischaemic stroke and neurodegenerative diseases were also excluded.

Surgical technique and postoperative treatment

Cataract surgery was performed in one eye of each patient by a single experienced surgeon (CET) at the Eye Clinic, S. Martino University Hospital of Genoa (Italy) under topical anaesthesia. The primary steps of the surgery were a self-sealing two-step temporal limbal microincision (2.2 mm), phacoemulsification in the capsular bag using the Infiniti surgical system (Alcon Novartis, Fort Worth, USA), and implantation of a foldable hydrophobic aspheric intraocular lens. Data regarding the microscope-light exposure time, the phacoemulsification time, the total volume of balanced salt solution used and the cumulative dissipated energy (CDE) were recorded at the end of all surgeries. Topical levofloxacin eye drops (Oftaquix, Santen Pharmaceutical Co, Osaka, Japan) were administered three times daily for 5 days postoperatively. Additionally, dexamethasone 1 mg/mL eye drops (Dexamono, Laboratoires Thea, Clermont Ferrand, France) were administered four times daily for the first postoperative week, after which the dosage was gradually tapered over the following 4 weeks.

In vivo confocal microscopy

IVCM (Heidelberg Retina Tomograph with the Rostock Cornea Module, Heidelberg Engineering, Heidelberg, Germany) was performed in all patients undergoing cataract surgery at baseline (visit 0 (V0); 7±1 days preoperatively) and 1 month after surgery (visit 1 (V1); 30±3 days postoperatively). In the control group, IVCM was performed during one routine eye examination (V0) and after 30±3 days (V1). Corneal SNP was evaluated by means of IVCM at the level of the corneal apex, as previously described.[11–13] Digital images were recorded with the sequence mode at a rate of three frames per second, including 100 images per sequence. A total of six to eight sequence scans of non-overlapping areas were recorded focusing on the SNP layer, typically at a depth of 50 to 80 µm. All IVCM examinations were performed by a single experienced examiner (RS). Three most representative images of corneal SNP were selected by two masked examiners (DS and LDC) based on optimal contrast, presence of in-focus nerves and absence of motion artefacts. Dendritic cells (DCs) density (cells/mm^2) was measured using the cell count software (Heidelberg Engineering GmbH) in the manual mode by identifying bright individual dendritiform structures with cell bodies in each image at the level of SNP. None of the patients instilled eyedrops within 2 hours before the ophthalmological measurements were performed.

Image analysis with ACCMetrics

IVCM images of corneal SNP were analysed with ACCMetrics (MA Dabbah, Imaging Science and Biomedical Engineering, Manchester, UK), which is a fully automated image analysis software, employing a machine-learning method.[14–16] The software was previously validated in the setting of corneal neuropathies comparing its performance with those obtained by both manual (CCMetrics) and semiautomated (NeuronJ) methods.[17] Representative IVCM images of one patient collected at V0 and analysed with ACCMetrics are shown in figure 1A,B.

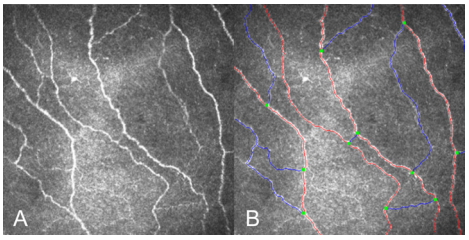

Figure 1 (A) Representative in vivo confocal microscopy image of corneal sub-basal nerve plexus obtained from the central cornea of a patient before surgery. All images are in the scale of 400×400 µm. (B) Automated image analysis using ACCMetrics software: main nerve fibres are indicated in red, nerve branches in blue and branch points in green.

The following seven parameters were calculated thanks to the automated software: (1) corneal nerve fibre density (CNFD), the total number of nerves/mm^2; (2) corneal nerve branch density (CNBD), the number of branches emanating from major nerve trunks/mm^2; (3) corneal nerve fibre length (CNFL), the total length of all nerve fibres and branches (mm/mm^2); (4) corneal nerve total branch density (CTBD), the total number of branches/mm^2; (5) corneal nerve fibre area (CNFA), the total nerve fibre area (mm^2/mm^2); (6) corneal nerve fibre width (CNFW), the average nerve fibre width (mm/mm^2); and (7) corneal nerve fractal dimension (CNFrD), a measure of the structural complexity of corneal nerves.[18]

The image analysis was applied to each of the three selected images by a trained masked examiner (FB). The average of the three values obtained for each parameter was used for statistical analysis.

Corneal sensitivity

Corneal sensitivity was evaluated in the central corneal region with the Cochet-Bonnet aesthesiometer (Luneau Ophthalmologie, Chartres, France) and values were recorded in millimetres per nylon filament length. All measurements were performed by the same investigator (DS) on all patients at V0 and V1 in both eyes.

Statistical analysis

The SPSS statistical software (SPSS) was used for data analysis. Values are expressed as mean±SD. The non-parametric Wilcoxon test was used to compare the seven IVCM parameters provided by the software ACCMetrics as well as the DCs density and corneal sensitivity at V0 and V1 in both operated and unoperated eyes. The correlations among IVCM parameters of corneal SNP, DCs density and corneal sensitivity values were evaluated using the Spearman's correlation analysis. The Bonferroni correction for multiple comparisons was used to confirm possible significance. A p value <0.05 was considered statistically significant.

RESULTS

Overall, 33 patients fulfilling the inclusion/exclusion criteria underwent cataract surgery during the study period. Of these,

Table 1 In vivo confocal microscopy parameters before (V0) and 1 month after cataract surgery (V1) in the surgery and fellow eye

	Operated eye			Unoperated eye		
Parameter	**V0**	**V1**	**P value**	**V0**	**V1**	**P value**
CNFD (n/mm^2)	21.21±6.56	15.35±7.00	**0.004**	23.20±7.26	20.11±6.69	**0.005**
CNBD (n/mm^2)	26.79±16.91	13.57±12.16	**<0.001**	29.76±15.25	24.28±14.88	**0.006**
CNFL (mm/mm^2)	13.49±3.42	9.67±3.44	**0.001**	14.02±3.82	12.53±3.60	**0.005**
CTBD (n/mm^2)	42.25±24.64	22.81±18.77	**0.002**	43.93±22.27	38.06±20.52	**0.049**
CNFA (mm^2/mm^2)	0.0058±0.0020	0.0040±0.0021	**0.002**	0.0057±0.0019	0.0049±0.0016	**0.009**
CNFW (mm/mm^2)	0.0209±0.0013	0.0207±0.0016	0.464	0.0205±0.0013	0.0206±0.0011	0.501
CNFrD	1.470±0.037	1.418±0.058	**0.001**	1.477±0.036	1.466±0.040	**0.020**

Bold typeface denotes p<0.05.
CNBD, corneal nerve branch density; CNFA, corneal nerve fibre area; CNFD, corneal nerve fibre density; CNFL, corneal nerve fibre length; CNFrD, corneal nerve fractal dimension; CNFW, corneal nerve fibre width; CTBD, corneal nerve total branch density.

30 patients (20 men and 10 women; mean age 74.7±8.6 years) completed successfully the study and their data were included in the final analysis. The remaining three patients were excluded because of loss to follow-up (n=2) and poor quality of IVCM images (n=1). Thirty age and sex-matched healthy subjects (18 men and 12 women; mean age 75.9±10.2 years) were included as controls.

All the surgeries of the patients included in the study were uneventful and surgical parameters showed a low variance among the operated eyes (always, p>0.05). In particular, the mean phacoemulsification time was 37.22±9.32 s, the mean balanced salt solution used was 94.76±27.39 mL, the mean CDE value was 8.49±2.89 s and the mean microscope-light exposure time was 11.03±3.03 min. Furthermore, no postoperative complications occurred over the 1-month follow-up.

Corneal sensitivity threshold did not differ significantly at V1 versus V0 in both eyes (respectively, 54.1±1.6 vs 54.8±0.9 mm in the operated eyes and 54.3±1.6 vs 54.7±1.2 in the unoperated eyes).

The mean values of the seven IVCM parameters of corneal SNP in patients undergoing cataract surgery collected at V0 and V1 and analysed with the software ACCMetrics are reported in table 1. Of these, CNFD, CNBD, CNFL, CTBD, CNFA and CNFrD significantly decreased 1 month after surgery compared with baseline values in both operated and unoperated eyes (always, p<0.049). Conversely, the mean value of CNFW did not change significantly from V0 to V1 in both operated and unoperated eyes (respectively, p=0.464 and p=0.501). No significant changes from V0 to V1 were found for any of the IVCM parameter in control subjects (always p>0.05). Representative IVCM images collected from both eyes of the same patient at V0 and V1 are shown in figure 2A–D .

Figure 3 shows the distribution of the patients according to the number of IVCM parameters that worsened after surgery in the unoperated eye.

The DCs density did not differ significantly at V1 compared with V0 in both operated and unoperated eyes (respectively, 21.6±18.2 cells/mm^2 at V1 vs 26.5±24.4 at V0, p=0.269 and 24.3±23.6 at V1 vs 20.9±19.0 at V0, p=0.182) as well as in control subjects (25.6±10.4 at V1 vs 26.2±11.6 at V0, p=0.515).

No significant correlations among IVCM parameters of corneal SNP, DCs density and corneal sensitivity values were found (always, p>0.05).

DISCUSSION

In the current study, we demonstrated that patients undergoing unilateral microincisional cataract surgery exhibit bilateral changes of corneal SNP 1 month postoperatively. These changes were subclinical and were detected only by IVCM examinations, while postoperative corneal sensitivity did not show significantly worse values in both operated and unoperated eyes compared with preoperative ones. The automated software employed in this study allowed a comprehensive morphometric analysis of all the characteristics of the corneal SNP. All IVCM parameters worsened significantly after cataract surgery compared with

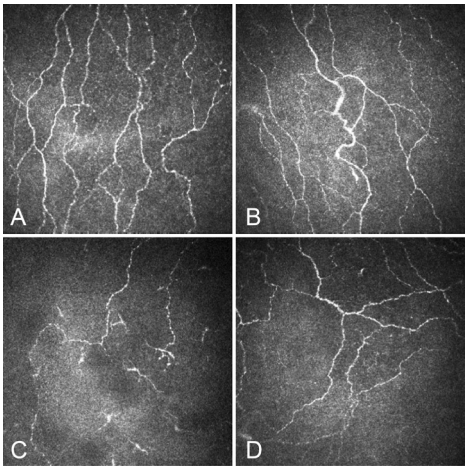

Figure 2 Representative in vivo confocal microscopy images of corneal sub-basal nerve plexus obtained from the central cornea of a patient before (A, B) and 1 month after surgery (C, D) in the operated eye (A, C) and in the contralateral unoperated eye (B, D). (A) ACCMetric analysis conducted at V0 in the operated eye calculated the following parameters: corneal nerve fibre density (CNFD)=37.5 n/mm^2; corneal nerve branch density (CNBD)=93.7 n/mm^2; corneal nerve fibre length (CNFL)=19.1 mm/mm^2; corneal nerve total branch density (CTBD)=137.5 n/mm^2; corneal nerve fibre area (CNFA)=0.011 mm^2/mm^2; corneal nerve fibre width (CNFW)=0.021 mm/mm^2; corneal nerve fractal dimension (CNFrD)=1.51. (B) ACCMetric analysis conducted at V0 in the unoperated eye calculated the following parameters: CNFD=18.7 n/mm^2; CNBD=50.0 n/mm^2; CNFL=14.7 mm/mm^2; CTBD=68.7 n/mm^2; CNFA=0.008 mm^2/mm^2; CNFW=0.021 mm/mm^2; CNFrD=1.45. (C) ACCMetric analysis conducted at V1 in the operated eye calculated the following parameters: CNFD=12.5 n/mm^2; CNBD=12.5 n/mm^2; CNFL=5.9 mm/mm^2 ;CTBD=6.2 n/mm^2; CNFA=0.004 mm^2/mm^2; CNFW=0.020 mm/mm^2; CNFrD=1.38. (D) ACCMetric analysis conducted at V1 in the unoperated eye calculated the following parameters: CNFD=6.2 n/mm^2; CNBD=12.5 n/mm^2; CNFL=12.5 mm/mm^2; CTBD=31.2 n/mm^2; CNFA=0.005 mm^2/mm^2; CNFW=0.019 mm/mm^2; CNFrD=1.46.

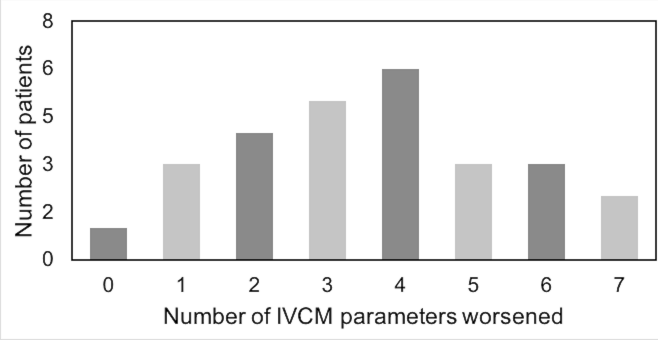

Figure 3 Column chart showing the distribution of the patients according to the number of in vivo confocal microscopy parameters that worsened 1 month after surgery in the unoperated eye.

baseline values in both operated and unoperated eyes, with the exception of CNFW. The measure of this parameter is of recent introduction and its value has been demonstrated to be higher in patients with dry eye and small fibre diseases, such as diabetic or sarcoidosis-associated neuropathies,[19 20] all conditions that were excluded in our study.

A previous study that evaluated SNP parameters in 30 healthy subjects with a mean age slightly lower compared with the present study reported better values of CNFD, CNFD, CNFL and CTBD compared with the results of the present study.[11] This finding confirms the evidence that corneal SNP is negatively affected by ageing, as reported by previous studies that reported a linear decline in subbasal nerve density ranging from 0.25% to 0.9% per year.[21 22]

One major limit of the use of IVCM for the detection of longitudinal changes after such treatment or surgery is related to the poor topographic reproducibility and the difficulty of ensuring the exact same location tested during follow-up examinations due to the lack of an active real-time eye-tracking system. This function would be able to eliminate the need for subjective operator placement in the desired sector of the cornea, thus increasing clinician ability to detect 'true' change over time rather than repeatability artefacts resulting from alignment error. In order to try to overcome this ineradicable drawback, we enrolled healthy matched control subjects who did not undergo surgery and examined them longitudinally. No significant changes from V0 to V1 were detected in any IVCM parameter for control subjects, thus suggesting a good reproducibility of IVCM examinations during follow-up imaging.

The topical treatment administered after cataract surgery may have influenced SNP changes occurring after surgery. Previous studies that investigated SNP in dry eye patients treated with topical corticosteroids showed a significant improvement of the morphology of sub-basal nerve fibres.[23 24] In the present study, despite the use of corticosteroids, a significant worsening of almost all IVCM parameters was detected in the operated eyes as a result of the detrimental effect of cataract surgery on SNP. Unlike corticosteroids, to the best of our knowledge, the effects of fluoroquinolones on corneal SNP are not known while a recent IVCM study described alterations at the level of corneal epithelium and stroma.[25]

The nervous interdependence between the two trigeminal nerves is not eye-specific and following peripheral nerve lesions in other regions of the body well-documented events that affect the opposite side non-lesioned structures have been described. These contralateral effects are qualitatively similar to those occurring at the ipsilateral side but are usually smaller in magnitude and have a briefer time course.[26] It is unclear whether these findings are an epiphenomenon or serve a biological purpose, but in either case the existence of these effects implies the presence of signalling mechanisms that link the two sides of the body at a central and/or peripheral level.[27]

To date, the presence of nervous and immune interdependence of affected and unaffected eyes has been described in the setting of neurotrophic and microbial keratitis.[5–8]It has been hypothesised that contralateral corneal nervous impairment could be related to neurogenic inflammation that occurs due to the local release of proinflammatory neuropeptides by afferent dysfunctional neurons.[5 28]

The bilateral effects on corneal SNP of unilateral surgery, herein demonstrated for the first time, open a new fascinating scenario to be explored in the setting of sequential cataract surgery with broad implications in the routine practice.

First, the integrity of corneal nerves is critical in maintaining the protective function of the cornea. Since corneal nerves influence signal transduction cascades involved in epithelial wound healing and homeostasis through the release of neurotrophic factors, their surgically induced impairment causes epithelial alterations and adversely affects the wound healing process.[29 30] In a health ocular surface system like that one of patients included in this study, these nervous alterations remain subclinical and detectable only by IVCM with no further detrimental effect on ocular surface. On the contrary, particular attention should be paid to patients at high risk for impaired wound healing such as diabetic ones, who represent a significant proportion of patients undergoing cataract surgery. However, it should be pointed out that in our study we excluded patients with diabetes or other central/peripheral neuropathies. A recent study showed that after cataract surgery patients with diabetes exhibited worse corneal nerve parameters in the operated eyes compared with patients without.[4] Although it is reasonable to hypothesise that a similar pattern could be detected also in the contralateral unoperated eye of patients with diabetes, future longitudinal studies in this subset of patients are required to confirm these findings.

Second, a common observation among ophthalmic surgeons is that patients with bilateral cataract undergoing sequential surgery often report a more unpleasant experience with their second surgery. The most accredited cause of this finding is usually attributed to psychological factors, such as mental stress, that contribute to the higher degree of pain reported by patients during the second eye surgery.[31]

The sympathetic alterations of corneal nerves of the unoperated eye detected in this study 1 month postoperatively (the most common interval time usually waited between first and second cataract surgery) may provide an organic cause for this phenomenon. In fact, it has been recently demonstrated in mice models that corneal injury determines an increase in ciliary nerve activity and may be responsible for ocular pain.[32]

Furthermore, it has been showed that the status of corneal nerve fibres influences the success rate of pharmacological therapy with anaesthetics in the setting of fibromyalgia. In particular, the subset of patients who presented corneal nerve abnormalities were found to show a poor analgesic response to anaesthetics.[33] We speculate that this scenario may occur also during the second cataract surgery, when the presence of corneal nerves abnormalities determined by the first operation may be responsible for the greater unpleasant experience and the higher discomfort symptomatology experienced.

Third, the contralateral eyes are usually used as controls in clinical practice and research studies, or as the so-called independent eyes to measure outcome parameters. The present

findings indicate that this practice should be rethought, because the comparison between affected and unaffected eyes may lead to confounding results.

Surprisingly, we did not find a significant increase of DCs in both operated and unoperated eyes 1 month postoperatively, and the DCs density remained within the wide physiological range described in the literature.[34] Corneal neuroimmune crosstalk links nervous dysfunction and inflammation activation through the anterograde nerve degeneration and the release of neuropeptides that increases the immune response of corneal tissue, thereby generating mature DCs. This phenomenon has been described in various chronic ocular surface conditions, such as infectious and inflammatory diseases.[35 36]

Several explanations can be made to support the discrepancy between nervous damage and inflammation activation found in our study. First of all, the distribution of DCs is uneven over the entire cornea and their density is reported to be maximum at the limbus, less in the peripheral cornea and least in the central cornea, the only region examined in our study by IVCM.[33] Second, it has been demonstrated that the recruitment of DCs happens as early as 24 hours after nerve injury; subsequently, the mature DCs migrate to the draining lymph nodes to activate effector lymphocytes. Therefore, as demonstrated also in other studies, the DC density returns to baseline values within 1 month after an acute injury.[35–37] Lastly, a possible confounding factor is related to the usage of topical corticosteroids in the operated eye in the first postoperative week since it has demonstrated that these therapies are able to reduce the density of DCs.[23 24] It can be speculated that, though not significant, the decrease of DCs density in the operated eyes could be caused by the effect of postoperative treatment with corticosteroids. Conversely, the not significant increase of DCs density in the unoperated eyes could be related to the sympathetic effect of cataract surgery in eyes that did not receive any anti-inflammatory therapy.

The corneal sensitivity threshold did not change significantly 1 month after surgery, in agreement with others that demonstrated its rapid decrease in the early postoperative period followed by a gradual improvement within 1 month postoperatively in healthy patients.[38]

The main limitation of this preliminary study is related to the small sample size and the limited follow-up that prevented us to assess the persistence or resolution of these changes over time as well as the long-term implications of the contralateral sympathetic nervous response. However, a recent research paper reported that the reduced number of ipsilateral sub-basal nerve fibres returned to normal values within 8 months after cataract surgery,[2] and this time interval could be considered reasonable also for the bilateral changes. The identification of the exact timeframe for the resolution of nerve abnormalities may help to suggest the most appropriate time interval to be waited between the first and the second operation in the setting of sequential cataract surgery. In addition, the postoperative treatment of the unoperated fellow eye with tear substitutes enriched with nutrients for nerves could be taken into account in order to support corneal nerve recovery.

In conclusion, patients undergoing cataract surgery exhibit bilateral subclinical alterations at the level of corneal SNP 1 month postoperatively. These alterations are likely caused by the sympathetic inflammatory response occurring at the level of the unoperated eye in the early postoperative period. If confirmed in larger subsets, this finding could have significant implications in the setting of sequential cataract surgery.

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Contributors GG, VS and AV designed the study. FG and FT recruited the patients. DS evaluated corneal sensitivity. RS made the in vivo confocal microscopy examination. DS and LDC selected the images. CET and RS did cataract surgery procedures. FB analysed the images with ACCmetrics. MP and CS did the statistical analysis. PV and CET supervised the work. GG wrote the draft. All the authors reviewed the manuscript.

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Patient consent for publication Not required.

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Data availability statement Data are available upon reasonable request. The data that support the findings of this study are available from the corresponding author (GG), upon reasonable request.

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