In Vivo Confocal Imaging of the Conjunctiva as a Predictive Tool for the Glaucoma Filtration Surgery Outcome

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Purpose. To examine the preoperative conjunctival dendritic cell density (DCD), goblet cell density (GCD), and stromal meshwork reflectivity (SMR) in glaucomatous patients undergoing filtration surgery, using in vivo confocal microscopy (IVCM).

Methods. Sixty-six patients were enrolled. At baseline, IVCM was performed at the site planned for surgery, and was repeated after 12 months at bleb site. Surgery was successful when a one-third reduction of baseline IOP was obtained at the last follow-up. The main outcomes were baseline DCD, GCD, and SMR, and 12 months IOP. The relations between baseline confocal parameters and 12 months IOP were analyzed.

Results. Filtration surgery was successful in 43 patients (group 1: complete success, 25; group 2: qualified success, 18), and unsuccessful in 23 patients (group 3). Baseline IOP (mm Hg) was 27.6 ± 2.8, 28.8 ± 4.1, and 27.7 ± 3.2 in groups 1 to 3, respectively. Preoperative DCD and SMR were lower in group 1 compared with groups 2 (P < 0.001, P < 0.05), and 3 (P < 0.001); preoperative GCD was higher in group 1 compared with groups 2 and 3 (P < 0.001). DCD and GCD were also different between groups 2 and 3 (P < 0.05, P < 0.001). At 12 months, IOP reduced by 43.5%, 38.4%, and 15.8% in groups 1 to 3. Twelve-month IOP reduction negatively correlated with baseline DCD and SMR (P < 0.001, r = −0.786; P < 0.05, r = −0.618), and positively with GCD (P < 0.001, r = 0.752).

Conclusions. Preoperative DCD, GCD, and SMR are parameters correlated with the filtration surgery outcome, with DCD presenting the strongest correlation. IVCM of the conjunctiva may represent an imaging tool to predict the surgical success in glaucoma.

Keywords: primary open-angle glaucoma, glaucoma filtration surgery, filtering bleb, conjunctiva, in vivo confocal microscopy

Filtration surgery, which still represents the most diffuse surgical procedure to control IOP in glaucoma, leads to the formation of an intrascleral fistula draining the aqueous humor (AH) from the anterior chamber into the subconjunctival space (filtration bleb). After reaching this space, AH is removed by different routes, such as the vascular or the trans bleb-wall routes. Because of this, the conjunctiva is considered the most important structure affecting the glaucoma filtration surgery outcome. It has been widely demonstrated that the development of an unbalanced fibrosis at bleb site during the postoperative period may hinder the AH resorption, resulting in inadequate IOP control.

Critical risk factors for bleb-wall fibrosis are represented by the long-term use of IOP-lowering medications, previous surgical manipulations of the conjunctiva, inflammatory ocular surface diseases, younger age, and the presence of profibrotic components in the AH filling the bleb cavity. All these conditions promote chronic inflammation of the conjunctiva, which deeply stimulates local myofibroblasts after surgery and leads to filtration failure. Unfortunately, the preoperative clinical examination of the ocular surface does not provide sufficient information about the inflammatory status of the conjunctiva and, thus, is inappropriate to predict surgery outcome.

Ocular surface biomarkers may overcome this limitation, guiding clinicians in assessing the risk of filtration failure in a more accurate way. Conjunctival gel-forming mucins, such as trefoil factor family 1 (TFF1) and MUC5AC, HLA-DR, goblet cells (GCs), and immuno-inflammatory cells, have been proposed as potential predictive indicators for filtration surgery outcome. Moreover, Chong et al.11 reported a correlation of preoperative tear levels of monocyte chemoattractant protein-1 (MCP-1) and propensity to bleb fibrosis. Nevertheless each of these parameters needs to be measured in an invasive way and reflects only in part the elements involved in postoperative filtration ability of the bleb.
In vivo confocal microscopy (IVCM) may provide rapid information of the most crucial conjunctival components that condition and are involved in the filtration ability of a bleb after surgery, in a noninvasive way.\textsuperscript{5,10,12–15} Dendritic cells (DCs), GCs, and the stromal fibrosis, which are indicators of inflammation, trans bleb-wall AH flow, and bleb-wall AH resisitivity, respectively, represent some of these key components.

Recently, the in vivo preoperative density of GCs has been reported to positively correlate with the surgical success in patients who are candidates for glaucoma surgery.\textsuperscript{10} These initial results stimulated the interest on IVCM as a useful tool to estimate the preoperative risk of failure, based on the conjunctival conditions.

The aims of the present study were to correlate the preoperative conjunctival dendritic cell density (DCD), goblet cell density (GCD), and stromal fibrous meshwork reflectivity (SMR), with the 12-month success in glaucomatous patients undergoing filtration surgery, using IVCM.

**METHODS**

**Patients**

This was a 12-month, prospective, single center, case-control study. Sixty-six consecutive patients (66 eyes) with uncontrolled primary open-angle glaucoma (POAG) and candidates to receive mitomycin-C augmented Ex-PRESS (Alcon Laboratories, Fort Worth, TX, USA) implantation were consecutively enrolled. Fifteen healthy subjects (15 eyes) were also enrolled and served as controls. The research adhered to the tenets of the Declaration of Helsinki and our institutional review board (Department of Medicine and Ageing Science of the University ‘G. d’Annunzio’ of Chieti-Pescara, Chieti, Italy) approved the study. Written informed consent was obtained from all subjects before enrollment, after explanation of the nature and possible consequences of the study.

Inclusion criteria for patients with glaucoma were as follows: best corrected visual acuity (BCVA) ≥8/10, diagnosis of POAG, uncontrolled IOP (>21 mm Hg, mean of three measurements acquired during a diurnal tonometric curve) under maximal tolerated medical therapy (including oral acetazolamide; therapy had to be unmodified during the past 3 months), central corneal thickness (CCT) between 550 and 580 μm, progression of the visual field (VF) damage confirmed on three consecutive examinations (Humphrey field analyzer [HFA] II 750; Carl Zeiss Meditec Inc., Dublin, CA, USA [30-2 test, full-threshold]). VF damage progression was assessed with the trend-based analysis of the HFA Guided Progression Analysis software: when the magnitude of visual field index slope was worse than 1% per year with a \( P \leq 0.05 \), the progression was considered clinically significant. In the case both eyes were eligible, the eye with the higher IOP or the more advanced perimetric damage (glaucoma staging system 2).\textsuperscript{16} was included in the study.

Exclusion criteria were the following: diagnosis of angle-closure, secondary or end-stage glaucoma; previous ocular surgeries, such as surgical procedures for ocular surface diseases, penetrating keratoplasty, retinal detachment surgery, or filtration surgery; indication for phaco-trabeculectomy or cataract surgery planned within 12 months thereafter; history of ocular diseases other than glaucoma; or systemic or topical therapies in the past 6 months potentially affecting the ocular surface status other than topical therapy for glaucoma, contact lenses, and pregnancy.

Healthy controls had to show a BCVA ≥8/10, mean IOP lower than 18 mm Hg, CCT ranging from 550 to 570 μm, absence of glaucomatous optic neuropathy, and normal VF and retinal nerve fiber layer thickness (Cirrus, version 6.0; Carl Zeiss Meditec) examinations. Exclusion criteria were a history of systemic or topical therapy, ocular or systemic diseases in the past 6 months, pregnancy, and contact lens wear.

**IVCM of the Conjunctival Confocal Parameters**

IVCM was performed to evaluate the DCD, GCD, epithelial microysts (EMs), and SMR. DCs appears as hyperreflective elements, with a different morphological feature according to their maturity and activation: mature cells present an elongated body with long membrane processes resembling nerve dendrites, whereas immature cells present a large body with rare membrane processes, if any. DCs can be observed within the epithelium (10–30 μm of depth) or within the close subepithelium (50–100 μm), isolated or in clusters, and work by modulating the immune response of the ocular surface toward external pathogenic stimuli.\textsuperscript{19–21} GCs are roundish cells located within the conjunctival epithelium (10–30 μm), filled with mucin granules, and with a peripherally displaced hyperreflective nucleus. They appear larger than surrounding epithelial cells, hyperreflective, singly dispersed or crowded in clusters, and produce the mucin components of the tear film.\textsuperscript{10,13,19,20,22} In addition, GCs have been proposed also as carriers of the AH through the bleb-wall epithelium in patients who underwent filtration surgery for glaucoma.\textsuperscript{3,10} EMs are...
particular attention to avoid bleb traumatisms in patients who
the mean microcysts density and area (MMD and MMA) taking
confocal microscopy was repeated at the bleb site to evaluate
the SMR.

depth, where are located most parts of collagen fiber bundles)
DCD and GCD, and from the deep stroma (50–150
epithelium and subepithelium (10–50
of the confocal session. Images were acquired from the
instructed to direct the gaze downward for the entire duration
after surgery, was analyzed. To explore this site, patients were
selected for the analysis. A single experienced IVCM operator
images without motion blur or compression lines were
acquired at the established areas and depths during baseline
and 12-month sessions, and 10 randomly selected high-quality
images without motion blur or compression lines were
selected for the analysis. A single experienced IVCM operator
performed the confocal examinations and selected the images
(LB); a second experienced operator evaluated the images
(VF). Both the operators were masked for the patient history
and for grouping.

round- or oval-shaped intraepithelial (10–30 μm) extracellular
structures, isolated or sometimes confluent or clustered, with
an inner hyporeflective feature. Sometimes they may host
hyperreflective elements inside, such as presumed inflamma-
tory cells or amorphous protein material. These structures
have been considered in vivo hallmarks of AH percolation
through the bleb-wall epithelium in successful filtration
surgery.6,10,14,15 The stroma of the conjunctiva presents a
superficial adenoid layer (immediately posterior to the
epithelium), which appears as a pale field with some collagen
fibers and scattered cells, and a deep dense fibrovascular
network with bundles of hyperreflective collagen fibers hosting blood
vessels.19 The definition and reference images of each confocal
parameter were consistent with those reported in the
literature.3,10,13–15,19–23

Confocal Microscopy Procedure
The technical characteristics of the laser scanning confocal
microscope (HRT III Rostock Cornea Module; diode-laser 670
nm; Heidelberg Engineering, Heidelberg, Germany), and the
details of conjunctival examination were reported else-
where.10,12,13,20 The device allows an automatic z-scan
determination of depth of focus within the conjunctiva,
provides a section thickness of approximately 10 μm, and a
lateral and transverse resolution of 4 μm.

At baseline (48–72 hours before surgery) a 5 × 5-mm area of
the upper bulbar conjunctiva, centered at 12 o’clock meridian
(2–4 mm from the limbus) and corresponding to the bleb site
after surgery, was analyzed. To explore this site, patients were
instructed to direct the gaze downward for the entire duration
of the confocal session. Images were acquired from the
epithelium and subepithelium (10–50 μm of depth) to evaluate
DCD and GCD, and from the deep stroma (50–150 μm of
depth, where are located most parts of collagen fiber bundles)
to evaluate the SMR.

After 12 months (or the last follow-up in failed cases),
confocal microscopy was repeated at the bleb site to evaluate
the mean microcysts density and area (MMD and MMA) taking
particular attention to avoid bleb traumatisms in patients who
underwent surgery.

Forty images (with a field of view of 400 × 400 μm) were
acquired at the established areas and depths during baseline
and 12-month sessions, and 10 randomly selected high-quality
images without motion blur or compression lines were
selected for the analysis. A single experienced IVCM operator
performed the confocal examinations and selected the images
(LB); a second experienced operator evaluated the images
(VF). Both the operators were masked for the patient history
and for grouping.

The Cell Count Software (Heidelberg Engineering GmbH) of
the confocal microscope was used to determine the number of
DCs and GCs (cells/mm² ± SD), and EM (cysts/mm² ± SD) in
manual mode; the surface area of EM (μm² ± SD) was
calculated using ImageJ software (http://imagej.nih.gov/ij/;
provided in the public domain by the National Institutes of
Health, Bethesda, MD, USA).

To quantify the confocal image reflectivity of the deep
stroma, we adopted a previously used arbitrary grading scale.23
The SMR was calculated by determining the average gray value
of the selected image using Image J software. This value
corresponded to the sum of gray values of all pixels in the
entire image divided by the number of pixels. An average gray
value less than 90.00 indicated a normal reflectivity (grade 0),
from 90.01 to 105.00 mild reflectivity (grade 1), 105.01 to
125.00 moderate (grade 2), and greater than 125.01 high
reflectivity (grade 3). Therefore, grades 0 to 3 corresponded to
a loosely, mildly, dense, and very dense arranged stromal
network, respectively (Fig. 1). The automatic brightness mode
was selected during examination. The confocal examination
lasted less than 5 minutes and no complications related to the
procedure were reported.

The primary outcomes of the study were the baseline DCD,
GCD, SMR, and 12-month IOP; the secondary outcomes were
the 12-month MMD and MMA. The correlations between
the baseline DCD with GCD and SMR; and the baseline DCD,
GCD, and SMR with 12-month IOP; the secondary outcomes were
reported.

Statistical Analysis
Analysis was performed by SPSS Advanced Statistical TM 13.0
Software and SPSS Sample Power Software (2005; Chicago, IL,
USA). Student’s t-test and χ² test were used to evaluate age,
IOP and sex differences between groups. Multivariate analysis
of variance was used to confirm the prognostic value of
variables among groups. Correlations among the variables were
determined using a nonparametric measure by the Spearman’s
index. A P < 0.05 was considered statistically significant.

RESULTS
Patients
Sixty-six patients successfully completed the study. Serious
intra- or postoperative complications were not reported in any
case: none of the patients were treated with topical steroids to
prepare the ocular surface for surgery or developed ocular
surface diseases after surgery.
6.43, 10–23 DCs were mainly found within the subepithelial
similar to those described in previous confocal studies (Fig. 1).
In Vivo Confocal Microscopy
Qualitative Data. DCs, GCS, and EMs presented features similar
to those described in previous confocal studies (Fig. 2).3,8,10,19–23
DCs were mainly found within the subepithelial
stroma or the basal epithelium. They were recognized both in
the mature (scleral nucleated cell body with associated
medium to long dendrites) and immature (large cell body with
fewer and shorter dendrites, or any) phenotypes dispersed
among epithelial cells or in the subepithelium at various
depths. In terms of morphology, no evident differences were
observed among groups 1 to 3; compared with healthy
subjects, glaucomatous patients displayed the typical branch-
ning phenotype of mature and activated cells. GCs were found
both in glaucoma patients and controls within the epithelium,
at 10 to 30 μm of depth, with the same morphological
characteristics. EMs presented different features: whereas in
healthy subjects they were small and scattered, with a
surrounding capsule, in the bleb-wall epithelium they were
larger, markedly hyporeflective, and frequently clustered or
coaescent. The deeper fibrous layer of the stroma was
recognizable in all subjects as a network of fiber bundles,
more evident and inhomogeneous in patients undergoing
surgery with respect to healthy controls (Fig. 2).
Clinical Data
Table 1 summarizes the demographic information, and baseline
and last follow-up clinical data. No significant differences were
found between glaucoma patients and controls for demograph-
ic parameters. Medical therapy was not different between
failed cases and controls. All successful cases reached the 12-
month follow-up, whereas the mean follow-up of failed
patients was 210 ± 50 days.
At baseline, IOP did not differ between glaucoma groups
but was significantly lower in controls ($P < 0.001$); at the last
follow-up visit, IOP was significantly reduced in groups 1 and 2
($P < 0.001$) without differences between them, whereas IOP
did not change in group 3 and controls. Surgical success
was obtained in 43 patients (65.2%), complete (group 1) in 25
patients, and qualified (group 2) in 18 (58.1% and 41.9%,
respectively); surgery was unsuccessful (group 3) in 23
patients (34.8%). The number of postoperative procedures
was significantly higher in group 3 compared with groups 1 and 2 ($P < 0.05$). At the last follow-up visit, IOP was reduced
by 43.3%, 38.4%, and 15.8% in groups 1 to 3, respectively,
and 2 ($P < 0.001$). At the last follow-up visit, IOP was significantly reduced in groups 1 and 2 ($P < 0.001$). GCD was
significantly lower in glaucomatous groups compared with
controls ($P < 0.001$), with values higher in group 1 compared
with groups 2 and 3 ($P < 0.001$) (Fig. 2). Group 2 showed DCD
and GCD values significantly different compared with group 3
($P < 0.001, P < 0.05$).
SMR was significantly higher in glaucoma groups compared
with controls ($P < 0.001$), with values higher in group 2
compared with controls ($P < 0.001$) and 2 ($P < 0.05$),
and higher in group 2 compared with group 1 ($P < 0.05$).
Conversely, no significant differences were found between
groups 2 and 3 (Fig. 2). Baseline parameters are reported in
Table 2.
At last follow-up, MMD and MMA were significantly higher
in functioning cases compared with failed cases and controls
($P < 0.001$), with values significantly higher in group 1
compared with group 2 ($P < 0.001$) (Fig. 2).
Correlations
Spearman’s correlation was used to test the strengths of
association between baseline confocal parameters and last
follow-up IOP, and the association between the number of
postoperative bleb management procedures and the IOP
reduction at the last follow-up.

### Table 1. Demography, Baseline, and Follow-Up Data

<table>
<thead>
<tr>
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<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Controls</th>
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<tbody>
<tr>
<td>No. of patients</td>
<td>25</td>
<td>18</td>
<td>23</td>
<td>15</td>
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<tr>
<td>Age, y ± SD</td>
<td>58.32 ± 6.85</td>
<td>59.72 ± 7.24</td>
<td>62.09 ± 8.73</td>
<td>60.40 ± 6.43</td>
</tr>
<tr>
<td>Sex, Male/Female</td>
<td>12/15</td>
<td>10/8</td>
<td>11/12</td>
<td>8/7</td>
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<tr>
<td>Duration of disease, mo ± SD</td>
<td>45.25 ± 12.32</td>
<td>48.71 ± 10.10</td>
<td>46.14 ± 11.53</td>
<td>-</td>
</tr>
<tr>
<td>Baseline IOP, mm Hg, mean ± SD</td>
<td>27.60 ± 2.8</td>
<td>28.88 ± 4.1</td>
<td>27.74 ± 3.2</td>
<td>15.35 ± 2.9*</td>
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<tr>
<td>Last follow-up IOP, mm Hg, mean ± SD</td>
<td>15.64 ± 1.81</td>
<td>17.79 ± 2.91</td>
<td>23.35 ± 3.5</td>
<td>15.18 ± 2.74</td>
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<tr>
<td>Baseline no. of drugs</td>
<td>3.15 ± 0.41</td>
<td>2.95 ± 0.39</td>
<td>3.25 ± 0.47</td>
<td>-</td>
</tr>
<tr>
<td>Last follow-up no. of drugs</td>
<td>1.80 ± 0.48‡</td>
<td>5.20 ± 0.50</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Baseline MD, dB, mean ± SD</td>
<td>-15.21 ± 3.26</td>
<td>-16.43 ± 3.32</td>
<td>-14.58 ± 4.12</td>
<td>2.15 ± 0.45*</td>
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<tr>
<td>Last follow-up MD, dB, mean ± SD</td>
<td>-15.96 ± 3.02</td>
<td>-17.12 ± 1.99</td>
<td>-15.98 ± 3.41</td>
<td>1.95 ± 0.25*</td>
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<tr>
<td>Postoperative procedures</td>
<td></td>
<td></td>
<td>0.91 ± 0.28</td>
<td>1.12 ± 0.31</td>
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</table>

MD, mean defect.
* $P < 0.001$ versus groups 1 to 3.
† $P < 0.05$ versus baseline and group 3.
‡ $P < 0.05$ versus group 3.
§ $P < 0.001$ versus groups 2 and 3.
|| $P < 0.05$ versus groups 1 and 2.
¶ Laser suture lysis and needling with 5-fluorouracil.

Quantitative Data. In the multivariate analysis, no significant
relations were found between age, sex, mean time
on therapy, and number of medications and the surgical
outcome. Conversely, analysis showed that DCD, GCD, and
SMR were significantly associated with the surgery outcome.

Baseline DCD was significantly higher in glaucomatous
groups compared with controls ($P < 0.001$), with values lower
in group 1 with respect to groups 2 and 3 ($P < 0.001$). GCD
was significantly lower in glaucomatous groups compared
with controls ($P < 0.001$), with values higher in group 1 compared
with groups 2 and 3 ($P < 0.001$) (Fig. 2). Group 2 showed DCD
and GCD values significantly different compared with group 3
($P < 0.001, P < 0.05$).

SMR was significantly higher in glaucoma groups compared
with controls ($P < 0.001$), with values higher in group 3
compared with groups 1 ($P < 0.001$) and 2 ($P < 0.05$),
and higher in group 2 compared with group 1 ($P < 0.05$).
Conversely, no significant differences were found between
groups 2 and 3 (Fig. 2). Baseline parameters are reported in
Table 2.
At last follow-up, MMD and MMA were significantly higher
in functioning cases compared with failed cases and controls
($P < 0.001$), with values significantly higher in group 1
compared with group 2 ($P < 0.001$) (Fig. 2).

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0.679; \( P < 0.001, r = 0.756 \). Mean number of postoperative procedures did not correlate with the IOP reduction in each group \((P = NS)\). Baseline IVCM parameters did not correlate with the number of medications, number of preserved eye drops and duration of treatment \((P = NS)\).

**DISCUSSION**

Besides demographic- and disease-related factors, the preoperative status of the conjunctiva represents an additional crucial factor for the outcome of filtration surgery.\(^4\),\(^1\),\(^8\),\(^1\),\(^2\),\(^4\),\(^2\),\(^5\) Because of the long-term use of IOP-lowering medications, the conjunctiva undergoes deep alterations that reach the highest expression when medical therapy fails and surgery is needed.

The most common epithelial changes are represented by squamous metaplasia, immuno-inflammatory cell infiltration, DC activation, and GC loss, whereas the collagen deposition represents the main stromal modification.\(^1\),\(^6\),\(^9\),\(^1\),\(^2\),\(^5\) All of these changes negatively affect the AH flow through the bleb wall after surgery.

IVCM has reached a consolidated position among imaging tools in ophthalmology, representing one of the elective techniques for ocular surface analysis. In glaucoma, IVCM proved essential to image tissue modifications induced by medical therapy and to evaluate the bleb functionality after filtration surgery.\(^5\),\(^6\),\(^1\),\(^2\),\(^1\),\(^5\),\(^2\),\(^1\),\(^2\),\(^1\)

In the present study, we found that in vivo imaging of the conjunctiva may predict the surgical outcome in patients who are candidates for filtration surgery. In detail, we observed that high preoperative levels of DCs, low levels of GCs, and a hyperreflective stroma at the site planned for surgery significantly increased the risk of bleb dysfunction and filtration failure. These parameters are an expression of the

<table>
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<th>Table 2. Baseline IVCM Parameters</th>
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<tr>
<td>DCD, Cells/mm(^2)</td>
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<tr>
<td>Group 1 87.44 ± 14.46(^\ast)</td>
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<td>Group 2 113.66 ± 16.65(\dagger)</td>
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<td>Group 3 135.04 ± 19.92</td>
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<td>Controls 24.15 ± 6.54</td>
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\( \ast P < 0.001 \) versus groups 2 and 3, and controls.
\( \dagger P < 0.05 \) versus group 2.
\( \ddagger P < 0.001 \) versus group 3.
\( \| P < 0.001 \) versus controls.
\( \| P < 0.001 \) versus group 3.
\( \| P < 0.05 \) versus group 3.
inflammatory status of the conjunctiva, the potential ability of
the epithelium in vehiculating AH after surgery, and the
stromal resistivity to the AH passage, respectively.\textsuperscript{3,5,10}
Therefore, they represent key structures because they directly
or indirectly affect the filtration ability of a bleb.\textsuperscript{4–6,25,30–32}
DCs are antigen-presenting cells that populate the entire
ocular surface and work by modulating the immune response
toward local stimuli. They were considered a hallmark of
inflammation in different ocular surface diseases (OSD),
including the glaucoma therapy-related OSD.\textsuperscript{19–21,23,26–28}
The high preoperative DCD observed in patients with a poor
surgical outcome was in line with numerous evidence, which
reported that an inflamed conjunctiva represents a strong risk
factor for bleb dysfunction.\textsuperscript{4,5,7,9,10,25,29,30}

Different OSDs, such as immunological disorders, infections,
chemical injuries, or the chronic instillation of topical
drugs, can cause stromal fibrosis.\textsuperscript{31} Between drugs, antiglau-
coma medications are well recognized promoters of collagen
deposition, because they increase TGF-β levels and stimulate
stromal myofibroblasts.\textsuperscript{4–6,25,30–32}

Our study supported this evidence, because a hyper-
reflective stroma (expression of fibrosis) was commonly
observed in patients under medical therapy compared with
healthy controls. Moreover, the significant positive correlation
between DCD and SMR supports that the iatrogenic inflam-
mation represents the promoter of the conjunctival scarring
processes. In terms of surgical outcome, a higher preoperative
stromal reflectivity negatively correlated with the IOP reduc-
tion, this suggesting that a thick and densely arranged stroma
before surgery reduces the AH filtration ability of a bleb. These
findings are in accordance with clinical studies that reported a
significant relation between preoperative fibrosis and filtration
failure.\textsuperscript{4,5,25,29,30,32}

Considering GCs, we found that high preoperative levels
of these cells were associated with a good surgical outcome. This
was also supported by the positive correlation between
preoperative GCD and postoperative MMD and MMA, which
are consolidated confocal markers of AH filtration through the
bleb wall. The significant relation between preoperative GCD
and the surgical outcome is in line with immunocytochemical
and confocal studies in which GCs and GC-derived mucins
were found to correlate with the filtration ability after
trabeculectomy.\textsuperscript{5,8,10} This evidence further supports the
already proposed theory that GCs are cytological carriers of
AH through the bleb wall.\textsuperscript{5,10}

Between confocal parameters, DCD presented the strongest
correlation with IOP reduction and, therefore, with surgical
success. This is not surprising, because inflammation repre-
sents the first step in the development of conjunctival changes
induced by drugs, and in initiating and maintaining bleb
scarring processes after surgery.\textsuperscript{7,14,23,25,29,30,53} This correla-
tion is also in line with numerous evidence reporting a robust
association between the surgical success and the number of
medications, the preoperative daily doses of benzalkonium
chloride (BAK), and the duration of treatment.\textsuperscript{25,29,34,35} In fact,
all these parameters act as strong inflammatory triggers.

The primary impact of preoperative conjunctival status on
surgical outcome seems also supported by the absence of
correlation between postoperative procedures and IOP reduc-
tion; this may rule out the possibility that the high success rate
in groups 1 and 2 could be related to a more intensive bleb
management.

As common experience in the daily practice, the clinical
observation of the conjunctiva or the use of routine ocular
surface tests does not accurately reflect the real tissue
conditions. Therefore, the only clinical assessment before
surgery cannot provide accurate information to define the best
time for surgery; to adopt the most appropriate measures
before, during, and after surgery, aimed at controlling the bleb
fibrosis; and to predict the final outcome.

In the attempt to overcome this limitation, different
molecular biomarkers, such as HLA-DR, GCs, MUC5AC, TFF1,
MCP-1, or immuno-inflammatory markers (CD3, CD4,
CD8, CD20, CD8, CD68) have been proposed over the years.\textsuperscript{5,8,10,29,35,36} However, all these biomarkers are indicators of
the epithelial inflammation and GC status.

Our results support the potential role of these molecular
biomarkers because the in vivo DCD and GCD can be considered
as epithelial indicators of the surgical outcome; in
addition, we introduced a stromal indicator of the conjunctiva,
which is the confocal reflectivity. In this way, to predict
surgical outcome, we proposed full-thickness, multiparametric,
in vivo imaging of the conjunctiva that concomitantly
considered all the elements conditioning the AH passage
through the bleb wall. In addition, with respect to ex vivo
techniques, IVCM permits imaging of all conjunctival layers in
a rapid, noninvasive, and cost-effective way.

The present study has some limitations. First, although GCs
and DCS are objectively measurable parameters, the stromal
reflectivity is an arbitrary index. Nevertheless, the selection of
the automatic brightness mode during the image acquisition,
and the calculation of the average gray value of the image
according to numeric values provided by ImageJ, significantly
reduce the arbitrariness of the method. Second, the study
cannot elucidate whether the DCD, GCD, and SMR before
medical therapy was different between groups. Thus, prospec-
tive studies following patients from initial diagnosis to surgery
are required. Third, given that the number of medications and
the duration of therapy before surgery were similar between
groups, we cannot clarify why group 1 presented higher levels
of GCD, and lower levels of DCD and SMR compared with the
other groups. Nevertheless, medical therapy–related factors
are the most probable candidates: in fact, the use of
preservative-free IOP-lowering formulations, associative fixed
combinations, lubricants, or short periods of steroids, could
have contained the OSD. Even though patient charts did not
methodically report this information, it could be hypothesized
that patients belonging to group 1 could have had a more
preserved ocular surface during medical management of
the disease. Fourth, there is also the possibility of normal
interindividual variability in the pool of DCs and GCs, and in
the stromal density of the conjunctiva, as previously pro-
posed.\textsuperscript{10,20}

Even if promising, at this stage, IVCM cannot be proposed
as a predictive imaging biomarker because validation steps
defining the sensitivity, specificity, and repeatability of
considered parameters in larger patient populations are
required. In addition, prospective confocal studies testing
the potential impact of the preoperative use of anti-
inflammatory agents in improving the conjunctival status
and the surgical outcome could further corroborate these
initial results.

In closing, our study confirmed that inflammatory conjunc-
tival alterations play a critical role in patients undergoing
filtration surgery. In this optic, strategies aimed at reducing
the ocular surface inflammation before surgery are strongly
recommended, because they may positively affect the bleb
functionality-related factors. Whether these results will be
validated, IVCM could be considered as an imaging tool to
stage the iatrogenic damage of the ocular surface, and the in
vivo confocal status of the conjunctiva proposed as a predictive
biomarker of the surgical outcome. This will guide clinicians in
determining the best time for surgery, and in adopting the most
appropriate perioperative strategies to contain the bleb
fibrosis.
Acknowledgments

Disclosure: R. Mastropasqua, None; V. Fasanella, None; L. Brescia, None; F. Oddone, None; C. Mariotti, None; S. Di Staso, None; L. Agnifili, None

References


