Optical Coherence Tomography Angiography Evaluation of Conjunctival Vessels During Filtering Surgery

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Purpose: To evaluate the changes in conjunctival vascularization with optical coherence tomography angiography (OCT-A) before and after filtering surgery and to correlate these results with filtering surgery success.

Methods: We evaluated 20 blebs of 20 patients after a first-time trabeculectomy. Conjunctival vascularization was quantified using ImageJ software. Eyes were classified into two groups according to the preoperative conjunctival vessel density: hypovascularized conjunctiva (HypoV; 10 eyes) and hypervascularized conjunctiva (HyperV; 10 eyes). The density of intraepithelial microcysts (0 to 3) was also analyzed.

Results: There were significantly more needling procedures in the HyperV group, with 70% of the eyes undergoing needling during follow-up compared to 20% in the HypoV group (P = 0.012). In the HyperV group, 50% of the eyes required IOP-lowering eyedrops after surgery, compared to 10% in the HypoV group (P = 0.029). HypoV showed significantly more intraepithelial microcysts than did HyperV at 1 week (1.1 vs. 0.4, P = 0.0215), 1 month (2.2 vs. 0.4, P = 0.0003), and 6 months postoperatively (2.0 vs. 0.7, P = 0.0068). A statistically significant correlation was found between preoperative conjunctival vascular density and mean IOP at 1 week (r = 0.483, P = 0.038), 1 month (r = 0.714, P = 0.001), and 6 months postoperatively (r = 0.471, P = 0.043). There was no statistically significant correlation between the preoperative conjunctival vascularization density and the eyedrop-year rate (r = 0.036, P = 0.8704) or the preservative-year rate (r = 0.1444, P = 0.5107).

Conclusions: Poor conjunctival vascularization was associated with lower IOP and a higher number of intraepithelial microcysts evaluated with OCT-A. OCT-A provides a simple, noninvasive, and reproducible method to analyze and quantify bleb vessels before and after filtering surgery.

Translational Relevance: Several studies have demonstrated that highly vascularized blebs might be associated with a higher risk of failure. OCT-A may provide a dye-free, noncontact method for monitoring conjunctival vascularization after filtering surgery.

Introduction

Glaucoma filtration surgery aims at decreasing intraocular pressure (IOP) by creating an alternative drainage route for aqueous humor from the anterior chamber to the subconjunctival and/or Tenon capsule, namely the filtering bleb. The development of this filtering bleb plays a critical role in the outcome of surgery. Failure of glaucoma filtration surgery is most commonly associated with an excessive fibrotic
response in the subconjunctival tissue, leading to filtering bleb scarring.\(^1\,^2\)

The postoperative evaluation of filtering blebs is essential in clinical practice during glaucoma surgery follow-up. Several clinical classification systems have been proposed to characterize bleb morphology based on slit-lamp examination.\(^3\,^5\) However, clinical evaluation of blebs can only indirectly evaluate the histologic changes that are responsible for the long-term success or failure of filtering surgery. The internal bleb morphology was first visualized using ultrasound biomicroscopy,\(^6\) confocal microscopy,\(^7\) and optical coherence tomography (OCT).\(^8,^9\) These techniques provided a better understanding of the internal structure of the bleb associated with their functionality. Recently, our group also analyzed filtering blebs using en face OCT to quantify intraepithelial microcysts, which are associated with lower IOP.\(^10\) Vascularization is also a determining factor in the success of the filtering surgery. In fact, angiogenesis is a central mechanism in the wound-healing process during the postoperative period after filtering surgery.\(^11\) Clinical,\(^12,^13\) in vivo confocal microscopy (IVCM),\(^7,^14,^16\) and angiographic studies\(^17,^18\) of conjunctival and episcleral vessels of filtering blebs have shown that highly vascularized blebs tend to be more frequently associated with surgical failure. Nevertheless, none of these imaging techniques provide a quantitative evaluation of the conjunctival vessels, and they require either contact with the eye (IVCM) or the injection of a dye (angiography).

OCT-angiography (OCT-A) is a revolutionary complement to the OCT to visualize moving elements of blood vessels without injection of a dye. OCT-A is a noninvasive technique that can easily be repeated over time to follow disease progression and monitor treatment efficacy. It was originally designed for imaging the retinal and choroidal vessels.\(^19\) However, in recent studies this imaging technique has also demonstrated its effectiveness in the qualitative analysis of anterior-segment vascularization, particularly corneal and limbal vessels. Recent studies on corneal and limbal neovascularization show that OCT-A can achieve noninvasive imaging of anterior-segment vessels and quantify vascular densities.\(^20\,^22\)

Recently, Yin X et al.\(^23\) have studied the relationship between filtering bleb vascularization and surgical outcomes after trabeculectomy. The aim of this study was to evaluate the changes in conjunctival vascularization with OCT-A before and after filtering surgery and to correlate these results with filtering surgery success.

### Methods

#### Patients

In this observational case series study, we included 20 filtering blebs of 20 patients who had undergone their first filtering surgery. The patients were consecutive cases followed at the Quinze-Vingts National Ophthalmology Hospital (Paris, France) for glaucoma. This study adhered to the tenets of the Declaration of Helsinki and was conducted at the Center For Clinical Investigations (CIC 1423) at the Quinze-Vingts National Ophthalmology Hospital, with the approval of the Institutional Review Board of Saint-Antoine University Hospital (CPP-Ile de France 5, number 10793). Informed consent was obtained from all eligible patients. All patients underwent trabeculectomy with intraoperative use of 0.02% mitomycin C for 2 minutes. The surgery was performed by one glaucoma surgeon. In all cases, limbus-based blebs were created. We used releasable scleral flap sutures. If needed, sutures were removed within the first 3 weeks using YAG laser. In case of subconjunctival fibrosis or encapsulation of blebs, needling with 5-fluorouracil (5-FU) was performed at the slit lamp using a 27-gauge needle. Patients who had combined cataract and glaucoma surgery or had undergone a second glaucoma surgery were excluded from this study.

#### Demographic and Clinical Data

The following demographic and clinical data were collected for each patient: age, sex, ethnicity, date of glaucoma diagnosis, visual acuity, cup/disc ratio, date of surgery, date of OCT-A examination, and preoperative and postoperative IOP at 1 week, 1 month, and 6 months using Goldmann applanation tonometry. Filtering blebs were analyzed according to the Indiana Bleb Appearance Grading Scale (IBAGS) criteria\(^4\) with slit-lamp photography: vascularization, height, horizontal extent, and the Seidel test. Therapeutic data were also collected: duration of treatment before surgery, type of medications, and the number of drops used daily. The presence of preservatives was also noted. As previously described by our group,\(^10\) “eyedrop-year” and “preservative-year” rates were calculated by multiplying the number of drops (number of preserved eyedrops) used daily by the duration of treatment (in years).
OCT-A Image Acquisition and Analysis

The anterior-segment OCT-A was performed by a single trained operator using the OCT RT with software (XR Avanti with AngioVue software; Optovue, Inc., Fremont, CA) preoperatively, at 1 week, 1 month (±2 weeks), and 6 months (±4 weeks) after surgery. The Avanti OCT detects blood flow in an acquired volume using the split-spectrum amplitude-decorrelation algorithm. It uses an 840-nm centered light source and is capable of 70,000 A-scans per second, which provide an axial resolution of 5 μm.

For the anterior segment, OCT-A images were obtained using the long corneal adapter (CAM-L), consisting of a lens that allows the production of 6×6-mm wide-angle images. OCT-A images of the blebs were taken while the patient was asked to look down and his or her upper eyelid was gently retracted to expose the entire bleb and the adjacent bulbar conjunctiva. For each bleb, three 6×6-mm acquisitions were performed in front of and around the scleral flap (nasal and temporal of the flap) (Figs. 1 and 2). At the same time, en face OCT images of the filtering blebs using the 3D cornea mode were performed (Fig. 3). For each bleb, six 4×4-mm acquisitions were performed in front of and around the scleral flap. The AngioVue software automatically recognized the surface of the filtering bleb. C-scans were automatically delimited by two parallel curved lines separated by an adjustable distance. These two lines could be moved from the surface (conjunctival epithelium) to the depth (sclera) to explore the different layers of the filtering bleb. The images were then evaluated qualitatively and quantitatively. Poor-quality images were defined as scans with signal strength less than 70; saccade or blinking artifacts disturbing vascularization analysis were excluded.

Qualitative Analysis

OCT-A acquisitions were first evaluated qualitatively by analyzing the conjunctival vasculature ranked from grade I (poor) to grade IV (hypervascularization) (ranking showed in Fig. 4). Then the number of intraepithelial microcysts was graded from grade 0 (none) to grade 3 (numerous) on bleb en face scans (Fig. 5) as previously described. Microcysts were defined as small, hyporeflective rounded spaces located at the epithelial surface of the bleb.

Quantitative Analysis

Conjunctival vasculature was quantified using ImageJ software (version 1.48; provided in the public domain by the National Institutes of Health, Bethesda, MD; http://www.imagej.nih.gov) (Fig. 6).

Angiograms were binarized to create black and white images with an autothreshold using the intermean algorithm. The density of binarized images represented the vessel surface density and was expressed as a percentage. It is a percentage of conjunctival vessels measured in a section of 6×6 mm among all the conjunctival tissue. A mean value of the three OCT-A images of each bleb was calculated. According to the conjunctival vessel density, we defined two groups: the hypovascularized conjunctiva group (HypoV), defined by a preoperative conjunctival vascular density less than 30%, and the hypervascularized conjunctiva group (HyperV), with a preoperative conjunctival vascular density greater than 30%. Binarized images were then automatically skeletonized to transform vessels into a 1-pixel-wide line as described by Zhang and Suen. The density calculated after skeletonization represented the vessel length density expressed in pixel⁻¹. After image binarization, skeletonization transforms vessels into a 1-pixel-wide line, occluding the width of the vessel. Therefore, the sum of the black pixels using skeletonized images corresponds to the total length of the vessels and the density measurements provide the vascular length density in mm⁻¹ or in pixel⁻¹. We excluded the corners of each scan from the analysis.

Figure 6 shows the binarization and skeletonization process of an OCT-A image of the conjunctiva.
Statistical Analysis

The results are presented as mean ± standard deviation for continuous variables and as percentages for categorical variables. A nonparametric Mann-Whitney test was used to compare continuous variables. For the binary results, the \( \chi^2 \) stratified test was used for the comparison of proportions between the groups. The Spearman coefficient was used to analyze the associations between two continuous variables. A \( P \) value < 0.05 was considered statistically significant.

Results

Patient Characteristics

This study included 20 eyes from 20 patients. There were 12 women and eight men with a mean age of 58.05 ± 13.26 years (range, 24–82 years). Sixteen patients had chronic open-angle glaucoma, three patients had chronic angle-closure glaucoma, and one patient had posttraumatic angle-recession glaucoma. The average follow-up was 7.7 ± 2.2 months. The HypoV group comprised 10 patients (10 eyes), and the HyperV group comprised 10 patients (10 eyes). Both groups were comparable in terms of demographic data except for ethnicity, with 20% of the patients of African descent in the HyperV group and none in the HypoV group. The HyperV group also had higher mean preoperative IOP of 32.6 [range, 18–59] versus 23.4 [range, 18–43] mm Hg in the HypoV group (\( P = 0.03 \)). There was no significant difference in the duration or severity of glaucoma or medical treatment between the two groups. Although not statistically
significant, there was a trend in terms of preservative-year rates: 18.92 [2–84] in the HypoV group versus 10.40 [0–30] in the HyperV group. All patients were treated with preoperative prostaglandin analogs with a significantly higher use of latanoprost in the HypoV group: 90% versus 40% (\(P = 0.01\)). The patients’ baseline characteristics are summarized in Table 1.

**Conjunctival Vessel Density**

A vascular density peak was observed 1 week after surgery (Fig. 7) with an average of 40.28% [21.47–51.63] and 9.23 px\(^{-1}\) [3.93–13.77], followed by a progressive decrease with a conjunctival vascular density at 6 months of 33.35% [12.35–36.79] and 7.92 px\(^{-1}\) [3.14–10.37]. The mean vascular density was still significantly higher in the HyperV group compared to the HypoV group at 1 week (44.10% [33.61–51.63] versus 33.92% [21.47–48.11], \(P = 0.0093\), \(\chi^2\) test), 1 month (38.20% [31.30–47.24] versus 25.25% [11.47–42.11], \(P = 0.0053\)), and 6 months after surgery (29.33% [20.06–36.79] versus 17.42% [12.35–27.66], \(P = 0.0002\)) (Table 2).

**Needling and Need for IOP-Lowering Medication**

There were significantly more needling procedures in the HyperV group, with 70% of the eyes undergoing needling during follow-up compared to 20% in the HypoV group (\(P = 0.012\)). In the HyperV group, 50% of the eyes required IOP-lowering eyedrops after surgery compared to 10% in the HypoV group (\(P = 0.029\)).

**Density of Intraepithelial Microcysts**

There were significantly more intraepithelial microcysts within the filtering blebs in the HypoV group compared with the HyperV group at 1 week (1.1 vs. 0.4, \(P = 0.0215\)) and 1 month (2.0 vs. 0.7, \(P = 0.0068\)). There was no statistically significant difference at 6 months postoperatively (1.1 vs. 0.7, \(P = 0.1537\)) (Table 2).

**Conjunctival Vessel Density and Preoperative Medical Treatment**

There was no statistically significant correlation between the preoperative conjunctival vessel density and the eyedrop-year rate (\(r = 0.173, P = 0.478\)) or the preservative-year rate (\(r = 0.07, P = 0.484\)).
Conjunctival Vessel Density and Microcyst Density

There was a statistically significant correlation between the preoperative conjunctival vascular density and microcyst density at 1 week ($r = -0.0603$, $P = 0.007$), 1 month ($r = -0.514$, $P = 0.026$), and 6 months after surgery ($r = -0.526$, $P = 0.022$).

Conjunctival Vessel Density and Needling or Use of IOP-Lowering Medication

There was a statistically significant correlation between preoperative conjunctival vascular density and the need for needling procedures at 1 month ($r = 0.4496$, $P = 0.0314$) and 6 months ($r = 0.595$, $P = 0.0027$). There was a statistically significant correlation between preoperative conjunctival vessel density

Table 1. Patient Characteristics

|                      | Hypovascularized Conjunctiva, $N = 10$ patients, 10 eyes | Hypervascularized Conjunctiva, $N = 10$ patients, 10 eyes | $P$  |
|----------------------|----------------------------------------------------------|----------------------------------------------------------|------|
| Age, y, mean [range] | 59.8 [24–82]                                              | 56.3 [45–67]                                              | 0.2861$^a$ |
| Sex                  |                                                          |                                                          |      |
| Female, n/N (%)      | 7/10 (70)                                                 | 5/10 (50)                                                 | 0.1940$^a$ |
| Male, n/N (%)        | 3/10 (30)                                                 | 5/10 (50)                                                 |      |
| African descent, n/N (%) | 0/10 (0)                                                | 2/10 (20)                                                 | 0.0839$^b$ |
| Type of glaucoma     |                                                          |                                                          |      |
| COAG, n/N (%)        | 8/10 (80)                                                 | 8/10 (80)                                                 | 0.5$^b$  |
| CACG, n/N (%)        | 1/10 (10)                                                 | 2/10 (20)                                                 | 0.2783$^b$ |
| Angle-recession glaucoma, n/N (%) | 1/10 (10)                        | 0/10 (0)                                                 | 0.1717$^b$ |
| Duration of glaucoma, y, mean [range] | 8.36 [2–15]                                      | 6.58 [1–14]                                              | 0.3476$^a$ |
| Preoperative IOP, mm Hg, mean [range] | 23.4 [18–43]                                    | 32.60 [18–59]                                            | 0.0307$^a$ |
| Cup/disc ratio, mean [range] | 0.8 [0.5–0.9]                                     | 0.7 [0.2–1]                                               | 0.1708$^a$ |
| Preoperative medical treatment |                                                      |                                                          |      |
| Eyedrop-year, mean [range] | 14.9 [2–45]                                     | 21.67 [2–84]                                              | 0.1361$^a$ |
| Preservative-year, mean [range] | 10.4 [0–30]                                      | 18.92 [2–84]                                              | 0.1032$^a$ |
| Treatment by prostaglandin analogs, n/N (%) | 10/10 (100)                                    | 10/10 (100)                                               |      |
| Latanoprost, n/N (%)  | 9/10 (90)                                                 | 4/10 (40)                                                 | 0.010$^b$  |
| Travoprost, n/N (%)   | 0/10 (0)                                                  | 1/10 (10)                                                 | 0.1717$^b$ |
| Bimatoprost, n/N (%)  | 1/10 (10)                                                 | 5/10 (50)                                                 | 0.029$^b$  |

$^a$ Mann-Whitney test.

$^b$ chi$^2$ test.

COAG, chronic open-angle glaucoma; CACG, chronic angle-closure glaucoma.
and IOP-lowering medication \((r = 0.4461, P = 0.0329)\) at 6 months but not at 1 month \((r = 0.3674, P = 0.0846)\).

**Preoperative Conjunctival Vessel Density and Mean IOP**

A statistically significant correlation was found between preoperative conjunctival vascular density and mean preoperative IOP \((r = 0.459, P = 0.05)\), mean IOP at 1 week \((r = 0.483, P = 0.038)\), 1 month \((r = 0.714, P = 0.001)\) and 6 months postoperatively \((r = 0.471, P = 0.043)\) (Fig. 8).

**Postoperative Conjunctival Vessel Density and Mean IOP**

There was no significant correlation between conjunctival vascular density measured by OCT-A at 1 week and mean IOP at 1 week \((r = 0.319, P = 0.182)\). A statistically significant correlation was found between conjunctival vascular density measured by OCT-A at 1 month and mean IOP at 1 month \((r = 0.550, P = 0.016)\). There was a significant correlation between conjunctival vascular density measured by OCT-A at 6 months and mean IOP at 6 months \((r = 0.546, P = 0.017)\) (Fig. 9).

**Table 2. Evolution of Microcyst Density in En Face OCT and Conjunctival Vascular Density in OCT-A During Follow-Up in Both Groups**

|                          | Hypovascularized Conjunctiva | Hypervascularized Conjunctiva | \(P^*\) |
|--------------------------|------------------------------|-------------------------------|--------|
| Microcyst density, mean  |                              |                               |        |
| Week 1                   | 1.1                          | 0.4                           | 0.0215 |
| Month 1                  | 2                            | 0.7                           | 0.0068 |
| Month 6                  | 1.1                          | 0.7                           | 0.1537 |
| Conjunctival vascular density, mean |                     |                               |        |
| Preoperative             | 16.24                        | 41.44                         | \(<0.0001\) |
| Week 1                   | 33.92                        | 44.10                         | 0.0093 |
| Month 1                  | 25.25                        | 38.20                         | 0.0053 |
| Month 6                  | 17.42                        | 29.33                         | 0.0002 |

\(^*\chi^2\) test.
Clinical Evaluation of Bleb Vascularity Using IBAGS and Mean IOP

There was no significant correlation between clinical evaluation of bleb vascularity using IBAGS at 1 week and IOP at 1 week ($r = 0.390, P = 0.1$). There was no significant correlation between clinical evaluation of bleb vascularity at 1 month and IOP at 1 month ($r = 0.377, P = 0.124$). A significant correlation was found between clinical evaluation of bleb vascularity at 6 months and IOP at 6 months ($r = 0.521, P = 0.024$) (Fig. 9).

Discussion

The long-term success of glaucoma surgery relies as much on postoperative follow-up as on the surgical procedure itself. In the literature, the success of trabeculectomy defined by an IOP $< 21$ mm Hg without the use of an IOP-lowering treatment at 2 years is approximately 80%, and the percentage of needling procedures is approximately 17%. This study showed that low preoperative conjunctival vessel density on the site of the future filtering bleb was associated with a lower IOP at 6 months with...
Figure 9. Comparison between clinical evaluation of bleb vascularity using IBAGS and conjunctival vascular density on OCT-A. (A) Correlation between clinical evaluation of bleb vascularity using IBAGS and IOP at 1 week. Spearman: $r = 0.390, P = 0.1$. Correlation between OCT-A evaluation of bleb vascularity and IOP at 1 week. Spearman: $r = 0.319, P = 0.182$. (B) Correlation between clinical evaluation of bleb vascularity using IBAGS and IOP at 1 month. Spearman: $r = 0.377, P = 0.124$. Correlation between OCT-A evaluation of bleb vascularity and IOP at 1 month. Spearman: $r = 0.550, P = 0.016$. (C) Correlation between clinical evaluation of bleb vascularity using IBAGS and IOP at 6 months. Spearman: $r = 0.521, P = 0.024$. Correlation between OCT-A evaluation of bleb vascularity and IOP at 6 months. Spearman: $r = 0.546, P = 0.017$. 

TVST | 2019 | Vol. 8 | No. 4 | Article 4
fewer needlings and less use of IOP-lowering medications. This has been demonstrated with both qualitative and quantitative methods based on a new noninvasive OCT-A technique.

These results are in accordance with several studies that have analyzed prognosis factors of filtering blebs. Shingleton\textsuperscript{28} showed that a highly vascularized bleb and the presence of corkscrew vessels were early signs of filtering surgery failure. Picht and Grehn\textsuperscript{3} studied five clinical parameters of filtering blebs to determine which one was associated with surgical success or failure: the presence of microcysts, density of vascularization, vessel shape, bleb size, and the presence of encapsulation. Signs for a functional bleb were the presence of microcysts, low vascularization, absence of corkscrew vessels, moderate height, and lack of encapsulation.\textsuperscript{3} Similarly, Sacu et al.\textsuperscript{12} studied the morphology of blebs and observed that patients who initially had corkscrew vessels 2 weeks after filtering surgery had a higher IOP at 1 year and developed more bleb encapsulation. IVCM studies have also shown that highly vascularized blebs are associated with more frequent failure of filtering surgery.\textsuperscript{7,14–16}

Our finding is similar to that of a recent study by Yin et al.,\textsuperscript{23} that found a peak in vessel density of the bleb 1 month after trabeculectomy using OCT-A. In this study, the vessel area density was also correlated with IOP 6 months post surgery, but preoperative vessel density was not studied. The peak of vessel density around the scleral flap was probably associated with scar formation in the surgical area during the first month. In our work, we studied vessel density not only around the scleral flap but also in nasal and temporal conjunctiva. However, none of these studies analyzed the predictive value of preoperative conjunctival density on filtering surgery success. Agnifili et al.\textsuperscript{29} and Mastropasqua et al.\textsuperscript{30} showed that markers of an inflammatory conjunctiva such as dendritic cells or goblet cell density and MUC5AC expression were correlated with the long-term success of surgery. Nevertheless, none of these authors quantified the conjunctival vessels before surgery.

In addition, we showed that microcyst density was significantly higher at 1 week, 1 month, and 6 months postoperatively in patients with low preoperative conjunctival vascular density. Studying filtering blebs with confocal microscopy, our group has shown that functional filtering blebs had many intraepithelial microcysts.\textsuperscript{6} More recently, by imaging filtering blebs with en face OCT, we also showed that a high density of microcysts was associated with better IOP control.\textsuperscript{9}

In our study, we showed a poor negative correlation between vascular density and microcysts may be because microcysts tend to be more rare after the first month and more difficult to measure due to fibrosis. It can also be explained by the small size of our cohort. We also suggest that microcysts can be either associated with an increase of vessel density (with lymphatic vessels) or the decrease of vascular density as we frequently measure them in functional filtering bleb.

On the other hand, we showed that the conjunctival vascular density in OCT-A reached a peak at 1 week after surgery. After filtering surgery in the scleral flap, the Tenon capsule, and the conjunctiva, a local inflammatory healing reaction takes place. The strength of the healing factors is sufficient to induce the formation of fibrotic tissue at the site of the surgery.\textsuperscript{1,31} It is therefore assumed that initial hyper-vascularization of the conjunctiva would enhance fibrosis because of the release of more proinflammatory factors from these blood vessels. Moreover, angiogenesis occurs during the wound-healing phase after glaucoma filtration surgery.\textsuperscript{11,31} It also corresponds to the time when sutures were lysed with YAG laser. This bleb manipulation could increase ocular surface inflammation, which could also explain the increase in vessel density within the first few weeks. Some authors have demonstrated that upregulation of vascular endothelial growth factor, a key mediator of angiogenesis, occurs in eyes that have undergone glaucoma filtration surgery.\textsuperscript{32,33} After the first month, we observed that the mean conjunctival vascular density in OCT-A decreased. Bouhenni et al.\textsuperscript{34} analyzed histologic sections of the blood and the lymphatic network of filtering blebs and showed that these structures were lower than that of the conjunctiva of healthy subjects. This observation could be explained by the intraoperative use of antimitotic agents such as mitomycin, which induces endothelial cell apoptosis.\textsuperscript{34}

We did not observe any statistically significant correlation between the preoperative conjunctival vascular density and the preservative-year or the eyedrop-year rates, although there was a trend for higher conjunctival vessel density in patients treated with preserved eyedrops. In these patients, administration of preservatives, especially benzalkonium chloride, may be responsible for chronic conjunctival inflammation, epithelial apoptosis or subconjunctival fibrosis and therefore a potential risk for early healing of the filtering bleb, which can lead to early encapsulation.\textsuperscript{35–37} The small number of patients in our cohort could explain the absence of a statistically
significant correlation between preservatives and vascular density. Furthermore, conjunctival hyperemia may be induced not only by preservatives, but also by the active ingredient of eyedrops. All the patients in the present study were treated with prostaglandin analogs prior to surgery. Hyperemia has been reported to occur in approximately 50% of patients treated with travoprost, between 15% and 45% of patients treated with bimatoprost, and between 5% and 15% of patients treated with latanoprost, as reported in product labeling. Among patients with preoperative conjunctival hyperemia, there was greater use of bimatoprost (50% vs. 18%) and less use of latanoprost (42% vs. 82%) compared to patients with a less-vascularized conjunctiva. Prostaglandin F2α causes vasodilation and increased release of nitric oxide from endothelial cells. Nitric oxide causes a relaxation of the muscle of the arteries or arterioles and thus has a vasodilatory effect. Clinical trials have found a higher incidence of conjunctival hyperemia in patients treated with tafluprost without preservative than in patients treated with tafluprost with preservative, thus showing the predominant role of the active ingredient in the appearance of this side effect.

One of the main limitations of our study is that the number of glaucoma patients included was small due to technical difficulties (eye movements or blepharospasm that induced many artifacts in the scans), and future studies with a larger number of patients are needed. Nevertheless, this study was a pilot study.

Another bias consisted of including patients of Afro Caribbean origins, and it is well established that rate of scarring and vascularization is different among different ethnicities; however, we defined our groups in terms of conjunctival vessel density.

We also noticed that the HyperV group had higher mean preoperative IOP than did the HypoV group. We suppose that this group had more severe glaucomas with a need for more aggressive lowering medications that induced more hyperemia.

These results should also take into account the technical limitations of anterior-segment OCT-A and the image analysis. Image acquisition artifacts are known factors that might limit the use of OCT-A. The most common artifacts are ocular movements and splitting of vessels. They appear when the software tries to correct eye movements. Image acquisition is difficult if the patient is not cooperating and cannot fixate because there is no eye tracking. In addition, if the patient has involuntary ocular movements, such as nystagmus or symptoms causing an abnormal blinking rate or blepharospasm, more artifacts will appear. The calculation of vascular density by binarization makes it possible to transform the vessels into white pixels on a black background and thus reduce the background noise represented by the artifacts in gray. However, it is necessary to choose a threshold that does not make vessels disappear or transform artifacts into vessels. Moreover, the current software automatically identifies the limits of the ocular surface that cannot be drawn manually. After surgery, the surface of the conjunctiva is often irregular, and the software can erroneously identify the limits of the conjunctiva and cause a discrepancy between the image and the curvature of the ocular surface. On conjunctival images obtained postoperatively or after a needling procedure, the presence of conjunctival hemorrhages was noted, which might mask conjunctival vessels and therefore lead to an underestimation of the vascular density measured by binarization. Finally, the reduced 6x6-mm scale allowed us to reconstruct the bleb around the scleral flap but did not allow us to obtain a large-field analysis of the entire conjunctiva. The small number of patients in the cohort is one of the main limitations of this study, which can be explained by the difficulty of making images without artifacts.

A previous study found that vascularization of a filtering bleb detected by OCT-A could be used for predicting surgery effect. Our results demonstrate that preoperative vascular density of the conjunctiva detected by OCT-A could also be used for predicting surgical success of failure.

A recent study on corneal neovascularization comparing anterior-segment OCT-A with indocyanine green angiography (ICGA) showed that OCT-A is a rapid and noninvasive method for measurement of the surface of corneal neovascularization. The limits of this examination adapted to the anterior segment are dominated by the absence of possible measurement of vascular flow, the superposition of several conjunctival vascular layers, and the need to take measurements by exporting images on external software. In addition, unlike fluorescein angiography or ICGA, the arterial and/or venous character of the vessels cannot be differentiated. In the present study, slit-lamp images of filtering blebs were produced to compare the clinically observed vascularization with OCT-A. Interestingly, conjunctival vascular density appeared to be much higher on OCT-A scans compared to slit-lamp examination. These could correspond to the episcleral or scleral deep subconjunctival network, but anterior-segment OCT-A did...
not allow us to sweep the entire conjunctival thickness. They could also correspond to vessels of a different nature: the lymphatics. The recent use of ICG-A to describe the lymphatic vessels of the cornea and limbus raises the possibility of applying OCT-A to detect lymphatics. Although reduced, flow is present in the lymphatic vessels. In 1960, Rayes et al.43 studied the distribution of the lymphatic vessels of the bulbar conjunctiva by injecting trypan blue into the various quadrants of the conjunctiva of 60 patients. More recently, new ICG lymphography techniques have been used to identify lymph nodes in the conjunctiva.44 A study carried out in 2012 on the conjunctiva of monkeys describes the lymphatic vascular network of the conjunctiva45: a superficial network under the conjunctival epithelium. This lymphatic vascular network might also play a role in the resorption of aqueous humor after filtering surgery.

OCT-A is a new imaging technique advancing en face OCT technology. Although no precise classification has yet been established, OCT-A examination of the anterior segment provides additional information to the clinical examination, in particular for the evaluation and quantification of vessels. For filtering surgery, OCT-A might provide a noninvasive evaluation of the density of conjunctival blood vessels that might be associated with the risk of failure. A better correlation was found between OCT-A findings and postoperative IOP results than that obtained with the IBAGS clinical bleb analysis system, suggesting that this new diagnostic technique might be promising in filtering surgery follow-up. A better preoperative evaluation of scarring risk factors would certainly help clinicians adjust their intraoperative antimetabolite regimen as well as the management of blebs during the follow-up of filtering surgery.

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