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Original Article

Confocal Microscopy of Filtering Blebs after Trabeculectomy

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Abstract

Introduction: Filtration surgery is the most effective method of lowering intraocular pressure (IOP) in patients with insufficient medical control. It consists in facilitating the drainage of the intraocular fluid (IOF) from the anterior chamber to the subconjunctival space and subsequent lowering of IOP. The formation of filtration blebs (FB) and the processes of scarring occurring in the conjunctiva are of particular importance in glaucoma surgery. In many cases, the appearance of FB does not match the IOP values, and what causes the failure after trabeculectomy often remains unclear. Often, over time, there is a change in the structure of the FB, as fibrous tissue grows, which prevents the IOF drainage. Laser scanning in vivo confocal microscopy is a non-invasive study allowing the production of layered images at the microstructural level with high resolution of both the cornea and other structures of the anterior ocular surface.

Aim: To evaluate the morphological structure and function of filtering blebs after trabeculectomy using in vivo confocal microscopy taking into account the type of implant and when the surgery was performed.

Materials and methods: The study included 33 patients, 46 eyes with glaucoma. Twenty-six of the eyes had primary open-angle glaucoma (POAG), 18 eyes had pseudoexfoliative glaucoma and 2 eyes had juvenile glaucoma. All patients underwent trabeculectomy with fornix-based flap, and three of the eyes underwent retrabeculectomy. Mitomicyn C (MMC) was administered intraoperatively to all patients. The study of the filtering bleb was performed by in vivo confocal microscopy (CFM) (Heidelberg Retina Tomograph II (HRT II) /Rostock Cornea Module/ (Heidelberg Engineering GmbH, Heidelberg, Germany), the period from trabeculectomy and examination being from 1 year to 22 years. An Express implant was placed in 14 eyes, Ologen implant in 7 eyes, and 25 eyes had no implant placed. In the analysis of the morphological structure of the filtering blebs, three indicators were evaluated: the type of epithelium, the type of stroma, and blood vessels.

Results: Statistical significance was established with regard to the function and morphological structure of the filtering bleb (p=0.009). Blebs with fine collagen mesh and dense collagen mesh demonstrate good function. In the case of blebs with insufficient function, those with a dense collagen network and hyper-reflective tissue predominated and there were no blebs with a fine collagen network, and in non-functioning blebs most common were those with a pronounced collagen network and hyper-reflective tissue. With regard to vascularization, we found that the functioning blebs in the shortest postoperative period were dominated by those with one blood vessel (stage 1) and there was no stage 3, with weak tortuosity, while in non-functioning blebs in the late postoperative period, there was moderate to severe vascularization and tortuosity (p=0.037), (p=0.043), (p=0.047), (p=0.021). The type of implant affects the tortuosity of the blood vessels of the filtering bleb (p=0.026). The blebs with Express implants show a slight tortuosity, followed by the blebs with Ologen implants. The highest percentage of highly kinked blood vessels occurred in blebs without an implant.

Conclusions: In vivo confocal microscopy is an innovative method which allows visualization of the internal structure of the filtering blebs at a cellular level, giving us a new insight into the ongoing healing processes, premising the function of the filtering blebs after glaucoma surgery.

Keywords

confocal microscopy, bleb, trabeculectomy, glaucoma

INTRODUCTION

Filtration surgery is the most effective method of lowering intraocular pressure (IOP) in patients with insufficient medical control. It is based on facilitating the drainage of the intraocular fluid from the anterior chamber to the subconjunctival space and subsequent lowering of IOP. The gold standard in glaucoma filtration surgery is trabeculectomy in which a filtering bleb is formed in the subconjunctival space where the intraocular fluid (IOF) drains.

In clinical practice, assessment of the filtering bleb (FB) after trabeculectomy is based on its appearance and IOP values. Through biomicroscopy, an examination and assessment based on area, height, vascularization, and the presence of cysts in its wall are performed.

A number of classifications have been developed, the most common in clinical practice being the Moorfields Bleb Grading System (MBGS) and the Indian Bleb Appearance Grading Scale (BAGS). The data, based on biomicroscopic criteria set by those classifications do not give us information about the morphology of the bleb at a depth.

The formation of FB and the processes of scarring occurring in the conjunctiva are of particular importance in glaucoma surgery. In many cases, the appearance of FB does not match the IOP values, and the reasons for failure after trabeculectomy often remain unclear. Often, over time, there is a change in the structure of the FB, as fibrous tissue grows, which prevents the IOF drainage. In practice, antifibrotic medicines such as mitomycin C (MMC), 5 fluorouracil (5 FU) or anti-VEGF agents are used to prevent fibrous tissue proliferation.

Confocal microscopy (CM) permits a morphological analysis of FB structure, visualizing microcysts in the epithelial layer of the conjunctiva, subepithelial tissue, blood vessels, tissue density and the presence of inflammatory cells.[1-4] Laser scanning in vivo confocal microscopy is a non-invasive tool allowing production of layered images at microstructural level with high resolution of both the cornea and other structures of the anterior ocular surface.

AIM

In the present study, we report the results of a retrospective study in patients after trabeculectomy with or without an implant, in whom the morphological structure and function of the FB were assessed taking into account when surgery was performed as well as age and sex.

MATERIALS AND METHODS

The study included 33 patients, 46 eyes with glaucoma. The mean age of the study group was 66.24±11.43 years, with an age range of 31-91 years. The relative share of both sexes was as follows: 45.5% men (N=15) and 54.5% women (N=18), without significant difference (p=0.623). Twenty-six of the eyes had a primary open-angle glaucoma (POAG), 18 eyes had pseudoexfoliative glaucoma, and 2 eyes had juvenile glaucoma.

All patients underwent trabeculectomy with a fornix-based flap according to the protocol, performed by two surgeons, with three of the eyes undergoing retrabeculectomy. MMC was applied intraoperatively for 2 minutes, using a small piece of surgical sponge soaked with 0.2 mg/ml MMC followed by copious irrigation of the operating field and the conjunctival flap at 0.9% NaCl. Express implant was placed in 14 eyes, Ologen implant in 7 eyes, and 25 eyes were without an implant.

All patients underwent a complete eye examination: examination of visual acuity, biomicroscopy of the anterior segment of the eye, examination of the fundus with 90D lens and tonometry and pachymetry with an air contactless tonometer.

The subject of the study was the epithelium and subepithelial tissue of the conjunctiva, as well as the blood vessels. The qualitative images without artefacts were carefully selected in order to perform qualitative analysis. Each eye was examined in less than 5 minutes and no complications related to the study were established.

The study of the filtering bleb was performed with an in-vivo confocal CM with Heidelberg Retina Tomograph II (HRT II)/Rostock Cornea Module/ (Heidelberg Engineering GmbH, Heidelberg, Germany), the period from trabeculectomy to examination varying from 1 year to 22 years. The device works with a diode laser at a wavelength of 670 nm, magnification of up to 800 times, lateral resolution of 1 μm and vertical resolution of 4 μm.

Immediately prior to the study, the topical anaesthetic proxymetacaine hydrochloride (Alcaine 0.5% collyre, Alcon) was applied to the lower conjunctival sac. Disposable PMMA applanation cap (Tomo cap), in which gel was used (Corneregel, Baush & Lomb GmbH, Germany) as a binding agent with the lens, was placed onto the lens of the microscope. The patient was positioned on the chinstrap and the headband of the apparatus, directing the gaze downwards (6h). The eyelid was raised manually without exerting any compression on the bulb, after which the applanation cap was taken to the area 3 mm above the upper limb in the area of the formed filtering bleb. The images were taken in 384×384 pixels with an area of 400×400 μm.

The following categories of filtering blebs were determined according to the values of IOP: 1) functioning – IOP≤18 mm Hg without any medication therapy; 2) insufficiently functioning – IOP<18 mm Hg with medication therapy; 3) non-functioning – IOP>18 mm Hg with medication therapy.

According to the postoperative period, the following groups were categorized: 1) up to 1 year after trabeculectomy; 2) from 1 to ≤3 years after trabeculectomy, and 3) over >3 years after trabeculectomy.

In the analysis of the morphological structure of the filtering bleb, three indicators were evaluated: the type of epithelium, the type of stroma, and blood vessels.

The choice of statistical methods was consistent with the objectives of the present study, the measurement scales of the data, as well as the distribution of the quantities. All continuous variables were checked for the presence/absence of normal distribution by the Kolmogorov-Smirnov test. Normal distribution was accepted when the Kolmogorov-Smirnov test showed a lack of statistical significance (p>0.05) and at values for asymmetry (skewness) within the permissible range (-1/+1) (George & Mallery, 2009). For continuous values with normal distribution, the analyses were performed via parametric statistical methods. Non-parametric statistical analyses were applied to the values measured on nominal, binary or ordinal scales. The following methods were used in the data analysis:

Parametric methods

- Mean values and standard deviation – descriptive statistics
- T-test for independent samples in the comparison of two groups (independent samples t-test)
- One-way analysis of variance (one-way ANOVA) for the comparison of more than two groups
- Tukey's post hoc test for inter-group comparisons in the presence of statistical significance by one-way ANOVA

Nonparametric methods

- Frequency analysis (number and %)
- Chi-square test to establish the association between variables measured on nominal or ordinal scales
- Fisher's exact test for comparison of proportions
- Binary logistic regression to establish the predictors of successful function of the blebs
- Odds ratio

RESULTS

With respect to the epithelium, the examined eyes were divided into four categories as follows: 31 without microcysts, 10 with optically empty microcysts, 4 with optically complete microcysts, and 1 with encapsulated microcysts **(Fig. 1)**.

Of the functioning blebs (n=15), 60% were without microcysts and 40% with optically empty microcysts. Of the insufficiently functioning blebs (n=11), 72.7% were without microcysts, 18.2% with optically empty microcysts, and 9.1% with optically complete microcysts. For non-functioning blebs (n=20), the distribution was as follows: 70% without microcysts, 10% with optically empty microcysts, 15% with optically complete microcysts, and 5% with encapsulated microcysts. Regardless of the function of the blebs, those without epithelial microcysts were predominant, therefore no significant association was found between the type of epithelium and the function of the blebs (p=0.290).

The distribution of epithelial species according to the postoperative period (≤1 year; 1-3 years; >3 years) was as follows: in the postoperative period ≤1 year, 6 blebs were categorized, of which 50% were without microcysts, 33.3% were with optical empty microcysts, and 16.7% were with encapsulated microcysts. In the postoperative period >1-3 years, 10 blebs were categorized, of which 80% were without microcysts and 20% were with optically empty microcysts. The remaining 30 blebs were observed in the postoperative period >3 years. Of these, 66.7% had no microcysts, 20% had optically empty microcysts, and 13.3% had optically complete microcysts. In all three postoperative periods, two epithelial types, without microcysts and with optically empty microcysts, predominated, with blebs without microcysts making up the majority in all three periods. Therefore, no significant association was found between the type of epithelium and the postoperative period of the blebs (p=0.136). A similar trend emerged between the different postoperative periods, but a higher percentage of epithelium without microcysts should be noted within the period 1-3 years.

Furthermore, no significant association was established between the epithelial type and the presence and/or type of implant (p=0.348). Blebs without microcysts predominated in all groups: 64% in the group without implants, 64.4% in the group with Express implants, and 85.7% in the group with Ologen implants. Optically empty microcysts were found in the group without implants (24%) and in the group with Express implants (28.6%). Optically complete microcysts were observed in a small percentage of the group without implants (12%) and in the group with Olo-

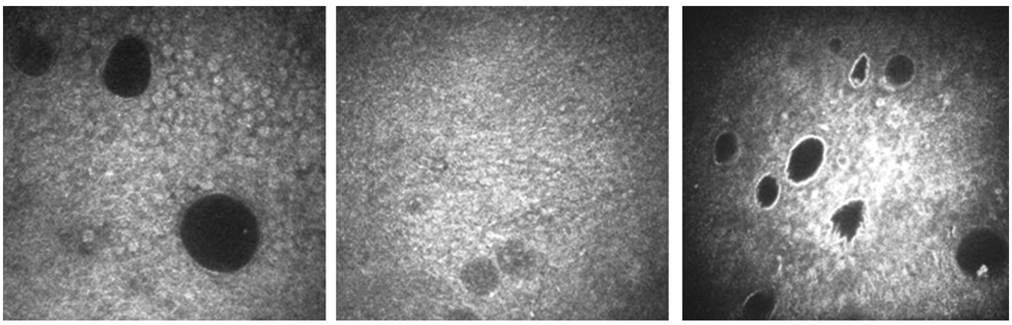

Figure 1. In vivo confocal imaging of conjunctival epithelium with optically empty microcysts, optically complete microcysts and encapsulated microcysts.

gen implants (14.3%). Encapsulated microcysts were found only in Express implants (7%).

The distribution of the blebs according to the morphological structure of the stroma is as follows: 8 blebs with a fine collagen network, 2 with a pronounced collagen network, 17 with a dense collagen network, and 19 with hyper-reflective tissue **(Fig. 2)**.

A significant relationship was established between the morphological types and the function of the blebs (*p*=0.009), which is explained by the following trends:

1) Two morphological types predominated in the functioning blebs: fine collagen network (40%) and dense collagen network (46.7%). Both types made up 86.7% of the functioning blebs and the remaining 13.3% were distributed between blebs with a pronounced collagen network (6.7%) and with hyper-reflective tissue (6.7%).

2) In the case of blebs with insufficient function, those with a dense collagen network (45.50%) and with hyper-reflective tissue (45.50%) predominated. In this morphological category, there were no blebs with a fine collagen network, and those with a pronounced collagen network made up only 10%.

3) In non-functioning blebs, those with hyper-reflective tissue predominated (65%). Blebs with a dense collagen network made up 25% of the non-functioning ones, with a fine collagen network only 10%, and there were no blebs with a pronounced collagen network.

We analysed and traced the relationship between the postoperative period and the morphological structure of the blebs. In the period ≤1 year, the blebs with a dense collagen network made up 50%, and those with hyper-reflective tissue – 33.3%. Blebs with a fine collagen network were only 16.7% and there were no blebs with a pronounced collagen network. In the period 1-3 years, the distribution of morphological species was as follows: 50% with dense collagen network, 30% with hyper-reflective tissue, 20% with fine collagen network and no blebs with pronounced collagen network. In the longest postoperative period >3 years, the trend was different from the previous two. In this period, the highest percentage was demonstrated by blebs with hyper-reflective tissue (46.7%), followed by those with a dense collagen network (30%). The percentage of blebs with a fine collagen network is similar to the previous two periods (16.7%) and only in this period were there blebs with a pronounced collagen network (6.7%). Despite some differences, no significant relationship was generally found between the morphological structure of the blebs and the postoperative period (*p*=0.827). In all three postoperative periods, blebs with a dense collagen network and hyper-reflective structure predominated.

We also examined the relationship between the morphological structure of the blebs and the absence or type of implant. In the group without implants, blebs with a dense collagen network accounted for 32% and those with hyper-reflective tissue – 48%. The other two species are rare, 16% with a fine collagen network and 4% with a pronounced collagen network. In the group with Express implants, 50% of the blebs were with a dense collagen network and 36% with hyper-reflective tissue. The other two types were established in only 7% of cases. In the group with Ologen implants, a different but statistically insignificant trend was set. In this type of implants, blebs with a fine collagen network predominated (42.9%), no blebs with a pronounced collagen network were observed, and the relative share of the other two types was 28.6%. In general, blebs of two morphological types predominated in all groups: with a dense collagen network and with hyper-reflective tissue, which explains the lack of significant association between the morphological structure of the blebs and the postoperative period (*p*=0.464). There was a slightly different trend in the group with Ologen implants, in which blebs with a fine collagen network predominated, but the difference was not significant. Due to the small number of blebs in the individual subgroups, it can be assumed that in a greater number of samples, more considerable differences are likely to be significant.

In relation to the cystic spaces, three categories were formed: without cystic spaces, cystic spaces without a capsule, and encapsulated cystic spaces. According to the function of FB, the distribution was as follows: in functioning FB: without cystic spaces (40%), without a capsule (46.7%), and with a capsule (13.3%), insufficiently functioning FB: without cystic spaces (54.5%), without a capsule (36.5%), and with a capsule (9%), non-functioning FB: without cystic spaces (60%), without a capsule (35%), and with a capsule (5%). No significant relationship was observed

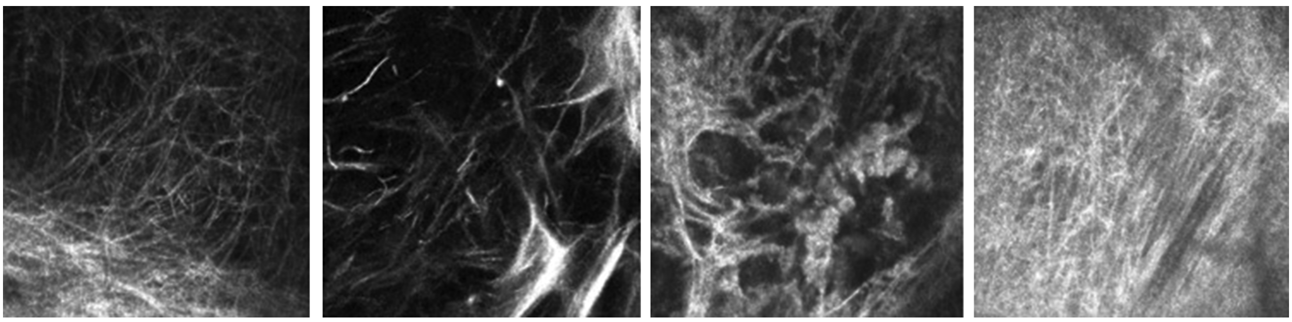

Figure 2. In vivo confocal imaging of conjunctival subepithelial tissue (stroma) – fine collagen network, pronounced collagen network, dense collagen network and hyper-reflective tissue.

between the function of the blebs and the cystic spaces (p=0.793).

According to the postoperative period and cystic spaces, the distribution was as follows: ≤1 year: without cystic spaces (83.3%), without a capsule (16.7%), and with a capsule (0%), 1-3 years: without cystic spaces (50%), without a capsule (40%), and with a capsule (10%), >3 years: without cystic spaces (46.7%), without a capsule (43.3%), and with a capsule (10%). There was also no significant relationship between cystic spaces and postoperative period (p=0.290), but it should be noted that even insignificant, the trend shown by blebs in the shortest postoperative period was different from that in the other two periods. In the period ≤1 year, the lack of cystic spaces reached the highest percentage (83.3%) in comparison to the other two periods, where besides the lack of cystic spaces, cystic spaces without a capsule were observed. Encapsulated cystic spaces were not observed in the shortest postoperative period having a low relative share in the other two periods. Despite the lack of statistical significance, in the shortest postoperative period there are a higher percentage of blebs without cystic spaces compared to the other two periods.

We also traced the relationship between cystic spaces and the presence of an implant. Without implant: without cystic spaces (48%), without a capsule (40%), and with a capsule (12%), with an Express implant: without cystic spaces (57%), without a capsule (36%), and with a capsule (7%), with an Ologen implant: without cystic spaces (57%), without a capsule (43%), and with a capsule (0%). No significant relationship was reported between cystic spaces and the presence of an implant (p=0.876). Regardless of the absence or type of implant, two categories predominated: without cystic spaces and cystic space without a capsule. Encapsulated cystic spaces are observed in a low percentage of eyes without an implant and such with an Express implant; they are totally absent in eyes with an Ologen implant.

According to the number of blood vessels, three stages were defined: stage 1 (10) includes the presence of 1 blood vessel; stage 2 (32) involves the presence of 2 to 3 blood vessels, and stage 3 (4) includes more than 3 blood vessels **(Fig. 3)**.

The relationship between blood vessels and bleb function was analysed using the chi-square test. A significant relationship was established between the number of blood vessels and the function of the blebs (p=0.037), which is mainly due to the higher percentage (40%) of functioning blebs with one blood vessel compared to the insufficiently functioning (9%) and non-functioning (9%) ones. In addition, the lowest stage 2 percentage was observed in functioning blebs (60%) compared to 82% in the insufficiently functioning blebs and 70% in the non-functioning ones. We should also note the absence of stage 3 in functioning blebs and its presence in non-functioning (15%) and insufficiently functioning (9%) blebs.

Furthermore, there was a significant correlation between the number of blood vessels and the postoperative period (p=0.043), ≤1 year: stage 1 (33.3%), stage 2 (66.7%), and stage 3 (0%); 1-3 years: stage 1 (40%), stage 2 (40%), and stage 3 (20%); >3 years: stage 1 (13%), stage 2 (80%), and stage 3 (7%). Two main trends can be observed: 1) In the shortest postoperative period (≤1 year), stage 3 is missing. 2) In the longest postoperative period (>3 years), the lowest percentage of stage 1 (13%) was established, as well as the highest percentage (80%) of stage 2. Overall, in the longest postoperative period, stages 2 and 3 accounted for 87%.

No significant relationship (p=0.226) was established while monitoring the relationship between the number of blood vessels and the type or absence of an implant. The distribution was as follows: without an implant: stage 1 (20%), stage 2 (72%), and stage 3 (8%); with an Express implant: stage 1 (36%), stage 2 (50%), and stage 3 (14%); with an Ologen implant: stage 1 (0%), stage 2 (100%), and stage 3 (0%). Nevertheless, it should be noted that with Ologen implants we established 100% presence of 2 to 3 blood vessels (stage 2) and absence of the other two stages. In the group with Express implant we reported the highest percentage of stage 1 (36%), while in the group with no implant stage 2 (72%) figured with the highest percentage.

The following distribution of blood vessels was found in terms of tortuosity: straight/unkinked (N=7), slightly kinked (N=16), medium kinked (N=10), and highly kinked (N=13). Regarding the relationship between tortuosity and the function of the blebs, the distribution was as follows – functioning IOP <18 mm Hg without therapy: straight – 34%, slightly kinked – 47%, medium kinked – 20%, and highly kinked – 0%; insufficiently functioning IOP <18 mmHg with therapy: straight – 0%, slightly kinked

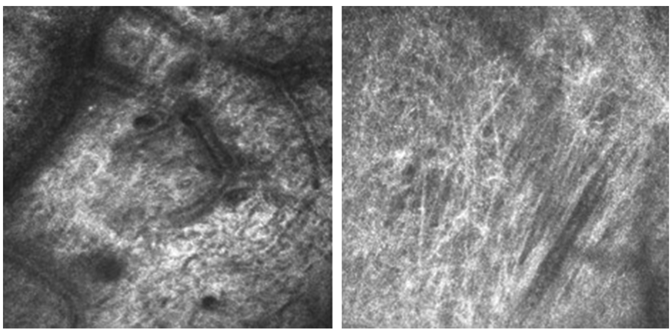

Figure 3. In vivo confocal imaging of conjunctival subepithelial tissue (stroma) – hyper-reflective tissue with blood vessels.

– 27.3%, medium kinked – 27.3%, and highly kinked – 45.5%; non-functioning IOP >18 mm Hg with therapy: straight – 10%, slightly kinked – 30%, medium kinked – 20%, and highly kinked – 40%. Between the two values a significant correlation was established (p=0.047) with the following trends: 1) the highest percentage of blebs with straight and slightly kinked blood vessels was observed in the functioning blebs (34%); 2) no highly kinked vessels were established in the functioning blebs; 3) with insufficiently functioning and non-functioning blebs, medium kinked and highly kinked blood vessels predominated.

While analysing the correlation between tortuosity and the postoperative period, a statistically significant relationship was established too (p=0.021). In the group ≤1 year: straight – 66%, slightly kinked – 17%, medium kinked – 17%, and highly kinked – 0%; 1-3 years: straight – 10%, slightly kinked – 50%, medium kinked – 20 %, and highly kinked – 20%; >3 years: straight – 6.7%, slightly kinked – 33.3%, medium kinked – 23.3%, and highly kinked – 36.7%. In the shortest postoperative period (≤1 year) the highest percentage of blebs with straight blood vessels (66%) and a lack of blebs with highly kinked vessels were established. Between 1-3 years, blebs with slightly kinked vessels predominated (50%), while in the latest period >3 years, blebs with medium and highly kinked vessels predominated (23.30% and 36.70%, respectively).

We also traced the relationship between tortuosity and the type of implant. Without implant: straight – 24%, slightly kinked – 24%, medium kinked – 16%, and highly kinked – 36%; Express: straight – 7.1%, slightly kinked – 64.3%, medium kinked – 14.3%, and highly kinked – 14.3%; Ologen: straight – 0%, slightly kinked – 14.3%, medium kinked – 51.1%, and highly kinked – 28.6%. The results show the presence of a statistically significant relationship (p=0.026), with the following trends: 1) in the absence of an implant, blebs with all stages of tortuosity were observed; 2) with Express implants, blebs with slightly kinked blood vessels predominated (64.3%); 3) with Ologen implants, blebs with moderately kinked blood vessels predominated (51.1%), there being no blebs with straight blood vessels; 4) the highest percentage of highly kinked blood vessels was observed in the absence of an implant (36%).

DISCUSSION

The performed analyses enabled us to establish statistical significance regarding the function and morphological structure of the filtering bleb. Blebs with fine collagen mesh and dense collagen mesh demonstrated good function. In the case of blebs with insufficient function, such with a dense collagen network and hyper-reflective tissue predominated, there being no blebs with a fine collagen network; and in non-functioning blebs, such with a pronounced collagen network and hyper-reflective tissue were the most common.

In the distribution of epithelial types relative to the function of the blebs, we did not establish any significant association between the type of epithelium and the function of the blebs (p=0.290). Regarding the epithelial type and the postoperative period and the presence of an implant, we did not detect any statistically significant association due to the predominant number of FB with epithelium without microcysts (p=0.136), (p=0.348), but a higher percentage of epithelium without microcysts in the postoperative period of 1-3 years must be taken into account. In contrast, other authors have reported a significant association when comparing the number and area of microcysts in the epithelium with functioning blebs.[3-5] Based on these data, we can assume that dense hyper-reflective stroma is an indication of poor FB function, confirming histopathologically that fibrotic scarring subconjunctivally is strictly associated with poor FB function.[3,6-9]

Although no significant correlation was established between the postoperative period and the type of stroma (p=0.827), the following trend can be observed: the greater the length of the postoperative period, the smaller the percentage of blebs with a dense collagen network and the higher the percentage of blebs with hyper-reflective tissue. In both samples mentioned above (Messemer, Caglar) the blurred type stroma occurs in the earliest postoperative period (first 2 months – Messemer) and (first 3 months – Caglar), while in our sample this type of stroma corresponds to hyper-reflective tissue, which is represented by the highest percentages in insufficiently functioning (45.5%) and non-functioning filtering blebs (65%). It must also be noted that in our sample the follow-up period starts from 1 year, so we have no data for such an early postoperative period (2-3 months). Based on these results, we can hypothesize that in the earliest postoperative period, the blurred type stroma is a predictor of poor function over time and corresponds to hyper-reflective (dense) tissue in the later postoperative period.

When tracking the relationship between the presence and type of implant and the type of stroma, we did not find any significant association (p=0.464), but a different trend emerged in the group of blebs with an Ologen implant, where a high percentage (42.9%) in favour of fine collagen network was established, but this difference did not reach significance. In larger samples, more considerable differences are likely to be significant.

After analysing the relationship between cystic spaces and the function of blebs, the postoperative period and the type of implant, we established no statistically significant associations in our sample. Unlike us, Messemer et al.[3] reported that the lack of encapsulated cystic spaces significantly correlated with good FB function (p=0.002).

As regards vascularization, we found that of the functioning blebs in the shortest postoperative period, those with one blood vessel (stage 1) predominated; stage 3 was missing; they demonstrated weak tortuosity. In contrast, non-functioning blebs in the late postoperative period showed a moderately to severely pronounced vascularization and tortuosity. Similar results have been reported by other authors[3,4,6,10,11], pointing out a significant correlation between the function and the number and the tortuosity of blood vessels. A lack or a small number of blood vessels with low tortuosity is characteristic of functioning blebs and vice versa, in non-functioning FB there is pronounced vascularization and tortuosity. In their sample, Morita et al. detected a difference in the degree of vascularization compared to the type of conjunctival flap in functioning FBs, reporting a higher degree of vascularization in the blebs with limbus-based conjunctival flap.[10]

The type of implant affects the tortuosity of blood vessels of the filtering bleb. Express implant blebs show slight tortuosity, followed by the blebs with an Ologen implant. The highest percentage of highly kinked blood vessels occurs in blebs without an implant.

In filtration glaucoma surgery, confocal microscopy provides some valuable data for assessing the function of the filtering blebs, by assessing the density of microcysts and the density of the stroma.[7] The preoperative status of the conjunctiva is also crucial for the outcome of the filtration surgery.[12] Prolonged medication therapy leads to profound changes in the conjunctiva, most commonly manifested with epithelial squamous metaplasia, immunoinflammatory cell infiltration, activation of dendritic cells (DCs), loss of Goblet cells (GCs), and increased collagen density. Mastropasqua et al.[12] report that increased dendritic cell density (DCs), low Goblet cell levels (GCs), and preoperative conjunctival hyper-reflective stroma are significantly high-risk factors for poor filtering bleb function and filtration surgery failure.

Preoperative medication therapy, as a risk factor for trabeculectomy failure, has been reported by other authors too.[13] The preservatives contained in the hypotensive ophthalmic preparations and beta blockers have a toxic effect, while prostaglandin analogues have a stimulating effect on the Goblet cells (GCs) in the conjunctiva. In patients who have undergone therapy with prostaglandin analogues and preservative-free medicaments, a higher level of Goblet cells is maintained.

CONCLUSIONS

In vivo confocal microscopy is an innovative method that allows visualization of the internal structure of the filtering blebs at a cellular level, giving us a new understanding of the ongoing healing processes which determine the function of the filtering blebs after glaucoma surgery. The method enables us to directly observe and track the histological processes and scarring mechanisms in the filtering bleb which correlate with filtration, providing us with the possibility of feasible specific treatment in order to increase the success of glaucoma filtration surgery. It also affords opportunities for reliable prediction and tracking of the function in relation to the morphological appearance of the blebs.

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Конфокальная микроскопия фильтрующих пузырьков после трабекулэктомии

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Резюме

Введение: Операция по фильтрации - наиболее эффективный метод снижения внутриглазного давления (ВГД) у пациентов при недостаточном медицинском наблюдении. Он состоит из поддержки оттока внутриглазной жидкости (ВГЖ) из передней камеры субконъюнктивального пространства и последующего снижения ВГД. Образование фильтрующих подушек (ФП) и процесс повреждения тканей конъюнктивы имеют особое значение в хирургии глаукомы. Во многих случаях возникновение ФП не совпадает со значениями ВГД, и причина неудачи после трабекулэктомии часто остается неясной. Часто со временем происходит изменение структуры ФП по мере роста фиброзной ткани, что препятствует оттоку ВГД. Конфокальная микроскопия с лазерным сканированием in vivo - это неинвазивное исследование, которое позволяет получать многослойные изображения на микроструктурном уровне с высоким разрешением роговицы и других структур передней поверхности глаза.

Цель: Оценить морфологическую структуру и функцию фильтрующих прокладок после трабекулэктомии с помощью конфокальной микроскопии in vivo с учётом типа имплантата и времени проведения операции.

Материалы и методы: В исследование включены 33 пациента, 46 глаз с глаукомой. У 26 глаз была первичная открытоугольная глаукома (ПОУГ), у 18 - псевдоэксфолиативная глаукома, у 2 - ювенильная глаукома. Всем пациентам была выполнена трабекулэктомия с использованием лоскута на основе свода, а на трёх глазах - ретробекулэктомия. Всем пациентам интраоперационно вводили Митомицин С (ММС). Исследование фильтрационной подушки проводили с помощью конфокальной микроскопии in vivo (КФМ) (Hedelberg Retina Tomograph (HRT) II / Rostock Cornea Module / Heidelberg Engineering GmbH, Гейдельберг, Германия), а период от трабекулэктомии и обследования составляет от 1 года до 22 года. Имплантат Expres был установлен в 14 глазах, имплантат Ologen - в 7 глазах и 25 глаз без имплантата. В анализ морфологического строения фильтрационных подушек были включены три показателя: тип эпителия, тип стромы и кровеносных сосудов.

Результаты: Статистическая значимость была обнаружена в отношении функции и морфологической структуры фильтрующей прокладки (p=0.009). Подушки с мелкой коллагеновой сеткой и плотной коллагеновой сеткой показали себя хорошо. В случае подушек с недостаточной функцией преобладали подушки с плотной коллагеновой сетью и гиперрефлективной тканью и отсутствовали подушки с тонкой коллагеновой сеткой, а в нефункционирующих подушках чаще всего встречались подушки с ярко выраженной коллагеновой сеткой и гиперрефлективной тканью. Что касается васкуляризации, мы обнаружили, что в функционирующих подушках в позднем послеоперационном периоде преобладали подушки с одним кровеносным сосудом (стадия 1) и не было стадии 3 с низкой складчатостью, в то время как в нефункционирующих подушках в позднем послеоперационном периоде наблюдались умеренные и тяжелые васкуляризация (p=0.037), (p=0.043), (p=0.047), (p=0.021). Тип имплантата влияет на складку кровеносных сосудов фильтрующей прокладки (p=0.026). Подушки Express слегка гофрированы, за ними следуют имплантаты Ologen. Самый высокий процент сильно складчатых кровеносных сосудов имел место в подушках без имплантата.

Заключение: Конфокальная микроскопия in vivo - это инновационный метод, который позволяет визуализировать внутреннюю структуру фильтрующих пузырьков на клеточном уровне, что даёт нам новое представление о текущих процессах заживления и определяет функцию фильтрующих пузырьков после операции по поводу глаукомы.

Ключевые слова

конфокальная микроскопия, пузырёк, трабекулэктомия, глаукома