A Comparison of Trabeculectomy Surgery Outcomes With Mitomycin-C Applied by Intra-Tenon Injection Versus Sponge

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• PURPOSE: To compare the outcomes of mitomycin-C (MMC) delivered by intra-Tenon injection vs sponge application during trabeculectomy surgery.

• METHODS: We retrospectively reviewed 566 patients with primary and secondary glaucoma diagnoses who received trabeculectomy surgery with MMC in an academic medical center. Exclusion criteria were age less than 18 years, no light perception vision, combined surgery, previous glaucoma incisional surgery, intraoperative 5-fluorouracil, or follow-up <1 month. Subjects were divided into 2 cohorts: MMC delivered by sponge application or by intra-Tenon injection. Main outcome measures were postoperative intraocular pressure (IOP) level and secondary measures were survival rate for IOP control, glaucoma medication use, complication rate, and vision.

• RESULTS: After inclusion/exclusion criteria, 316 eyes were available for analysis; 131 eyes had MMC delivered via sponge and 185 eyes via injection. Mean postoperative IOP was not significantly different between treatment groups but change in IOP from baseline was lower in the sponge vs the injection group 24 months after surgery ($P = .038$). The MMC sponge group had significantly more tense, vascularized, or encapsulated blebs as a late complication ($P = .046$). Time to failure for postoperative IOP control was not significantly different between MMC treatment groups, but older patient age and limbus-based conjunctival incision were associated with significantly longer time to fail.

• CONCLUSIONS: The application of MMC by injection was similar to application by sponge in lowering IOP in patients with glaucoma and the safety of both techniques appears to be comparable. Limbus-based conjunctival incision had longer time to failure for postoperative IOP control vs fornix-based incision. NOTE: Publication of this article is sponsored by the American Ophthalmological Society. (Am J Ophthalmol 2020;216:243–256. © 2020 Elsevier Inc. All rights reserved.)

TRABECULECTOMY WAS FIRST DESCRIBED IN 1968 when Cairns[1] reported a procedure in which a portion of the canal of Schlemm was removed along with adjacent trabecular meshwork to lower intraocular pressure (IOP). The original intention of the procedure was not to create a filtering bleb, but it was recognized that one was necessary to achieve pressure lowering. This surgery was subsequently modified by Watson and Barnett,[2] who created a scleral flap over an area in which sclera, cornea, and trabecular meshwork were removed to create a fistula into the anterior chamber. The enemy of trabeculectomy success is the body's own propensity to heal an open wound, and glaucoma surgeons have tried—and continue to try—various methods of retarding the wound-healing response. Examples of these methods are radiation, antimetabolites, amniotic membrane, anti–vascular endothelial growth factor, growth factor inhibitors, and bioengineered implants.[3]

One of the first antimetabolites used in trabeculectomy surgery was 5-fluorouracil (5-FU), which is a thymidylate synthase inhibitor that blocks the synthesis of thymidine, a nucleoside required for DNA synthesis.[4] By doing so, DNA synthesis is interrupted and in trabeculectomy surgery, fibroblast proliferation is inhibited. The Fluorouracil Filtering Surgery Study Group performed a prospective randomized trial in which 213 eyes were randomized to trabeculectomy performed with and without 5-FU.[5] The application of 5-FU was intense and it was delivered twice daily for the first 7 days and then once daily from day 8 to day 14. The use of this antimetabolite conferred a significant advantage, with 73% success rate for IOP control vs 50% in eyes that did not receive 5-FU during trabeculectomy surgery. Adverse events associated with 5-FU use included corneal epithelial cell toxicity, although visual acuity outcomes at 1 year were similar between groups. The use of 5-FU in trabeculectomy surgery was soon

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TABLE 1. Baseline Characteristics of the Study Populations

Baseline Characteristics	Sponge Group N = 131	Injection Group N = 185	*P* Value
Study eye, n (%)			
Right	65 (50)	95 (51)	.82
Left	66 (50)	90 (49)	
Sex, n (%)			
Male	55 (42)	97 (52)	.07
Female	76 (58)	88 (48)	
Age (mean ± SD)	64.32 ± 12.56	65.13 ± 11.93	.57
Ethnicity, n (%)			
White	74 (57)	101 (55)	.40
African American	23 (18)	32 (17)	
Asian	11 (8)	11 (6)	
Hispanic	14 (11)	16 (9)	
East Asian	5 (4)	9 (5)	
Unknown	4 (3)	16 (9)	
Glaucoma diagnosis, n (%)			
Primary open-angle glaucoma	88 (67)	138 (75)	.41
Primary angle-closure glaucoma	17 (13)	15 (8)	
Pigmentary glaucoma	3 (2)	7 (4)	
Mixed-mechanism glaucoma	1 (1)	2 (1)	
Normal-tension glaucoma	10 (8)	6 (3)	
Juvenile open-angle glaucoma	1 (1)	2 (1)	
Traumatic glaucoma	1 (1)	1 (1)	
Pseudoexfoliation glaucoma	7 (5)	6 (3)	
Other	3 (2)	8 (4)	
Medical history, n (%)			
Diabetes	27 (21)	34 (18)	.67
Hypertension	61 (47)	88 (48)	.91
Hypercholesterolemia	26 (20)	67 (36)	.002
Coronary artery disease	17 (13)	12 (7)	.07
Asthma	8 (6)	12 (7)	1.00
Rheumatoid arthritis	1 (1)	4 (2)	.41
Obstructive sleep apnea	1 (1)	0 (0)	.42
Depression	2 (2)	8 (4)	.20
Cerebral vascular accident	0 (0)	1 (1)	1.00
No medical problems	47 (36)	58 (31)	.47
Prior glaucoma procedures, n (%)			
Laser trabeculoplasty	52 (40)	48 (26)	.010
Iridotomy	18 (14)	12 (7)	.034
Iridoplasty	2 (2)	1 (1)	.57
None	66 (50)	125 (68)	.002
Lens status, n (%)			
Phakic	101 (77)	143 (77)	1.00
Pseudophakic	30 (23)	42 (23)	
Baseline IOP (mm Hg), mean ± SD	23.2 ± 8.3	20.7 ± 8.1	.010
Baseline medications, mean ± SD	2.5 ± 1	2.8 ± 1	.030
Preoperative visual acuity (logMAR), mean ± SD	0.24 ± 0.34	0.3 ± 0.46	.22
Conjunctival incision, n (%)			
Fornix-based	29 (22)	94 (51)	<.0001
Limbus-based	102 (78)	91 (50)	
Surgeon			
1	69 (53)	131 (71)	.001
2	62 (47)	54 (30)	
Length of follow-up (months)			
Mean ± SD	28.3 ± 15.2	23 ± 15.3	.003
Range	0.7-80.2	0.9-62	

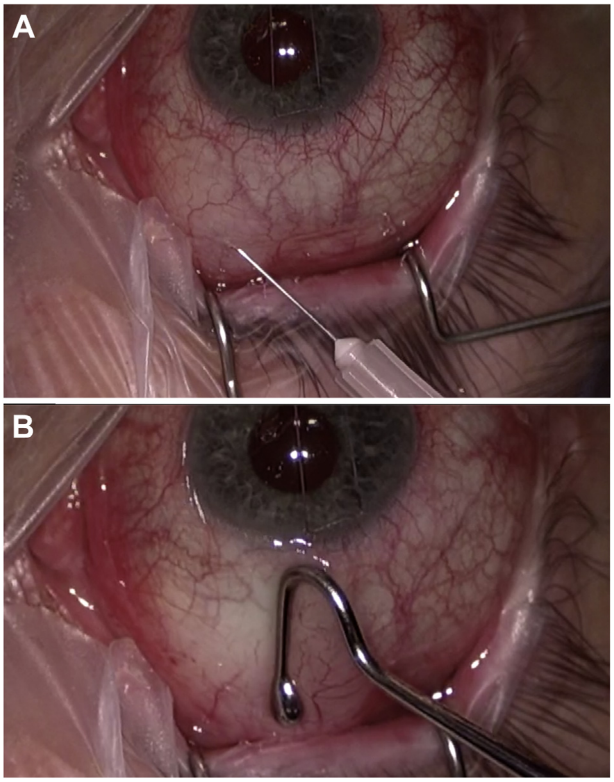


FIGURE 1. (A) Intra-Tenon injection of mitomycin-C during trabeculectomy surgery. The injection is approximately 9 mm posterior to the limbus and away from the superior rectus muscle. (B) A muscle hook is used to spread the mitomycin-C diffusely across the superior conjunctiva and Tenon layer.

followed by the use of another type of antimetabolite, mitomycin-C (MMC). This compound exists as a beautiful purple crystal that was first isolated in 1956 by Hata and associates, from the actinobacteria *Streptomyces caespitosus* in Japan.[6] The investigators recognized that this compound had an anticancer effect in animal models, and eventually MMC was approved in 1974 by the U.S. Food and Drug Administration as a treatment for lung and pancreatic cancers.[7] MMC causes alkylation, which leads to the cross-linking of DNA strands to interrupt the cell cycle.[8] It inhibits the proliferation of fibroblasts and endothelial cells, and its use in promoting bleb survival in trabeculectomy surgery by retarding the wound healing response was first reported by a group of Taiwanese ophthalmologists in 1990.[9] In this case series of 59 eyes in human subjects, Chen and associates[9] applied MMC with gel foam sponges at a concentration of 0.1 to 0.4 mg/mL underneath conjunctiva and Tenon layer. The MMC-soaked gel foam sponges were replaced every minute for 5 minutes. The success of IOP control <21 mm Hg with a mean of 3 years of follow-up was 77.8%.

Since 1990, the acceptance and use of MMC in trabeculectomy surgery has steadily increased in the United States, as demonstrated by a survey of American Glaucoma Society members conducted in 2016.[10] This survey found that the percentage of glaucoma surgeons using MMC with trabeculectomy surgery rose from 45% in 1996 to 97% in 2016. The advantage of MMC in relation to 5-FU is the ease of intraoperative application and its more profound effect on retarding fibroblast proliferation. The disadvantage of MMC is the creation of thin, avascular trabeculectomy blebs that are prone to leaks, which can in turn lead to the development of blebitis and endophthalmitis.[11–13] The incidence of late-onset endophthalmitis in eyes receiving trabeculectomy has been reported as 0.2%-1.5% without antimetabolite, 1.0%-5.7% with 5-FU, and 0.3%-4.9% with MMC.[3] To avoid these thin, leaky blebs, glaucoma surgeons have tried various methods of applying MMC during trabeculectomy surgery in the hopes of maintaining good IOP control while creating an ideal bleb morphology,[9,14–16] which may be described as a diffuse, low-profile, minimally vascularized structure.[17] Techniques to promote such a bleb, such as applying MMC over a wider area during trabeculectomy surgery, have been studied. In rabbit eyes, a wider application of MMC delivered by sponge reduced the formation of a cystic bleb and produced more diffuse, less vascularized blebs with prolonged survival.[18] In human eyes, it has been postulated that a fornix-based conjunctival approach allows for a more diffuse application of MMC delivered by sponge and that this may lead to more diffuse blebs.[17] Jones and associates also found that limbus-based trabeculectomy surgery resulted in a higher rate of cystic blebs (90%) in comparison to fornix-based conjunctival incision (29%).[17] Alternate methods of MMC delivery that result in diffuse application of the drug may be beneficial to bleb morphology as well, and may also promote better long-term IOP control. In 1 study, investigators applied amniotic membrane soaked in MMC under and over the trabeculectomy flap.[14] The IOP decreased from a preoperative mean of 32.2 mm Hg to 16.4 mm Hg after a mean of 9.8 months of follow-up. The authors reported no devastating complications such as suprachoroidal hemorrhage, endophthalmitis, or late hypotony.

Another novel method of applying MMC diffusely is by injection or irrigation of MMC into the Tenonlayer; this has been studied in animal[19,20] and in human[21–23] eyes. In 1 study using rabbit eyes, MMC applied by injection into conjunctiva was compared to 3 other methods: application with a regular surgical sponge, a scleral shield, and a presoaked soft contact lens. The investigators found the highest ocular tissue concentration of MMC in the subconjunctival injection group; the other 3 groups had similar concentrations. In 2008, Lee and associates[22] assessed the outcomes of MMC applied by intra-Tenon injection in human eyes. This was a noncomparative, retrospective study that included 46 eyes receiving combined cataract and trabeculectomy surgery and 62 eyes receiving trabeculectomy alone. At 1 year, the mean IOP was 12.2 ± 3.9 mm Hg in the group with combined surgery and

TABLE 2. Intraocular Pressure and Medication Use for Each Mitomycin-C Delivery Group

	MMC Sponge Group		MMC Injection Group		
	N	Mean (SD)	N	Mean (SD)	*P* Value
Mean IOP (mm Hg)					
Preoperative baseline	131	23.2 (8.31)	185	20.7 (8.1)	.0095
Month					
1	125	13.1 (7.06)	185	11.8 (5.22)	.09
6	108	11.8 (6.25)	151	10.5 (4.51)	.08
12	94	11.7 (5.78)	122	10.9 (4.58)	.34
24	84	11.0 (4.59)	92	11.6 (4.67)	.38
Change in IOP from baseline (mm Hg)					
Month					
1	125	10.1 (9.64)	185	8.9 (9.04)	.29
6	108	11.0 (7.88)	151	10.5 (8.43)	.62
12	94	10.8 (8.34)	122	9.9 (8.57)	.47
24	84	10.9 (7.35)	92	8.5 (8.03)	.038
Medications					
Preoperative baseline	129	2.5 (1.04)	185	2.79 (1.03)	.030
Month					
1	126	0.08 (0.37)	185	0.07 (0.39)	.84
6	108	0.13 (0.41)	152	0.28 (0.75)	.044
12	95	0.34 (0.77)	123	0.41 (0.89)	.54
24	84	0.42 (0.79)	92	0.60 (1.13)	.22

IOP = intraocular pressure; MMC = mitomycin-C.

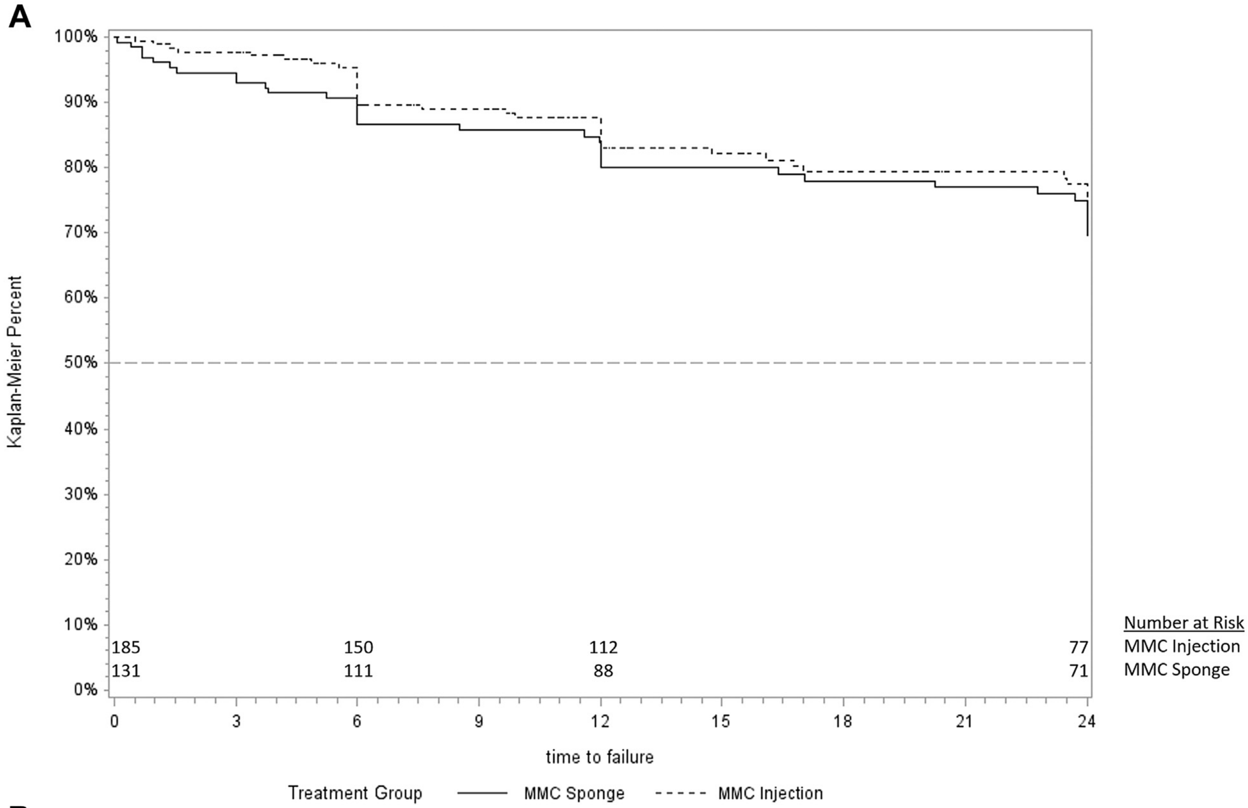
11.9 ± 3.6 mm Hg in the trabeculectomy-only group. The unqualified success rate of IOP control <21 mm Hg was 86% and 90% for the 2 groups, respectively, and complications such as hypotony (21.3%) and choroidal detachment (15.7%) were reported at rates similar to those seen in other published clinical trials involving trabeculectomy outcomes.[24–26] The authors concluded that the application of MMC by injection was an "effective technique." However, the limitations of this study were that it did not compare the injection technique to the more traditional sponge technique, the study was retrospective, and the sample size of trabeculectomy-only eyes was small. Following this publication, 2 short-term (6-12 months) studies[21,27] describing the outcomes of MMC application by subconjunctival injection (or by irrigation) vs MMC application by sponge for trabeculectomy surgery were performed. These studies showed no difference in mean postoperative IOP, while a third study, comparing MMC delivered by irrigation under conjunctiva and Tenon layer vs sponge application for trabeculectomy surgery with EX-PRESS shunt, showed a significant difference in change in IOP from baseline at month 6 for the MMC irrigation group. The first 2 studies revealed no significant differences in postoperative complications whereas the third study reported a significantly greater rate of hypotony (IOP <4 mm Hg) in the MMC irrigation group. Although these studies are helpful in assessing alternative methods of applying MMC during trabeculectomy surgery, they are all short term (6-12 months of follow-up), and longer-term data may reveal different outcomes.

In the present study, we compared long-term surgical outcomes of MMC delivered by intra-Tenon injection vs the more traditional sponge method in eyes undergoing trabeculectomy. We hypothesize that the diffuse application of MMC by injection may lead to lower postoperative IOP.

METHODS

THE HUMAN SUBJECTS REVIEW COMMITTEE (INSTITUtional review board) at the University of California, Davis approved a retrospective study protocol to evaluate all patients who had undergone a trabeculectomy surgery with antimetabolite performed by 2 faculty surgeons (M.C.L. and J.D.B.) at the University of California, Davis Medical Center. This research study is Health Insurance Portability and Accountability Act compliant and adheres to the tenets of the Declaration of Helsinki. Subjects were identified by reviewing billing CPT codes for trabeculectomy over a 13-year period. Inclusion criteria included patients with primary and secondary glaucoma diagnoses (Table 1), who were at least 18 years of age or older at the time of surgery and who had undergone primary trabeculectomy surgery with MMC with either a fornix-based or limbus-based conjunctival incision approach. Exclusion

A



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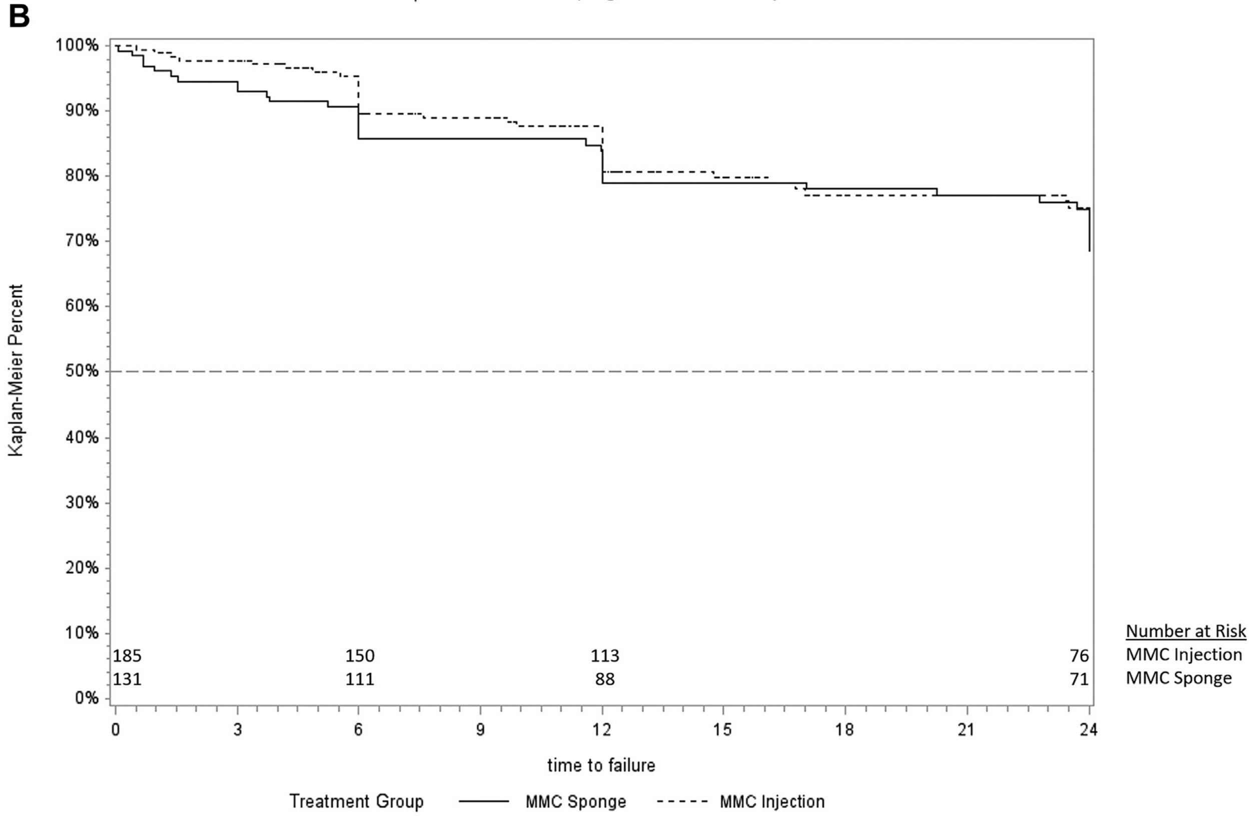

FIGURE 2. Kaplan-Meier estimates for the survival times of intraocular pressure (IOP) control (with or without medication) for different mitomycin-C (MMC) application techniques. Success was defined as (A) IOP $<$22 mm Hg and $>$5 mm Hg and IOP reduced by 20% or greater from baseline without additional glaucoma surgery and without a devastating complication (no light perception vision, endophthalmitis) and at lower IOP thresholds (B) $<$18 mm Hg and (C) $<$15 mmHg.

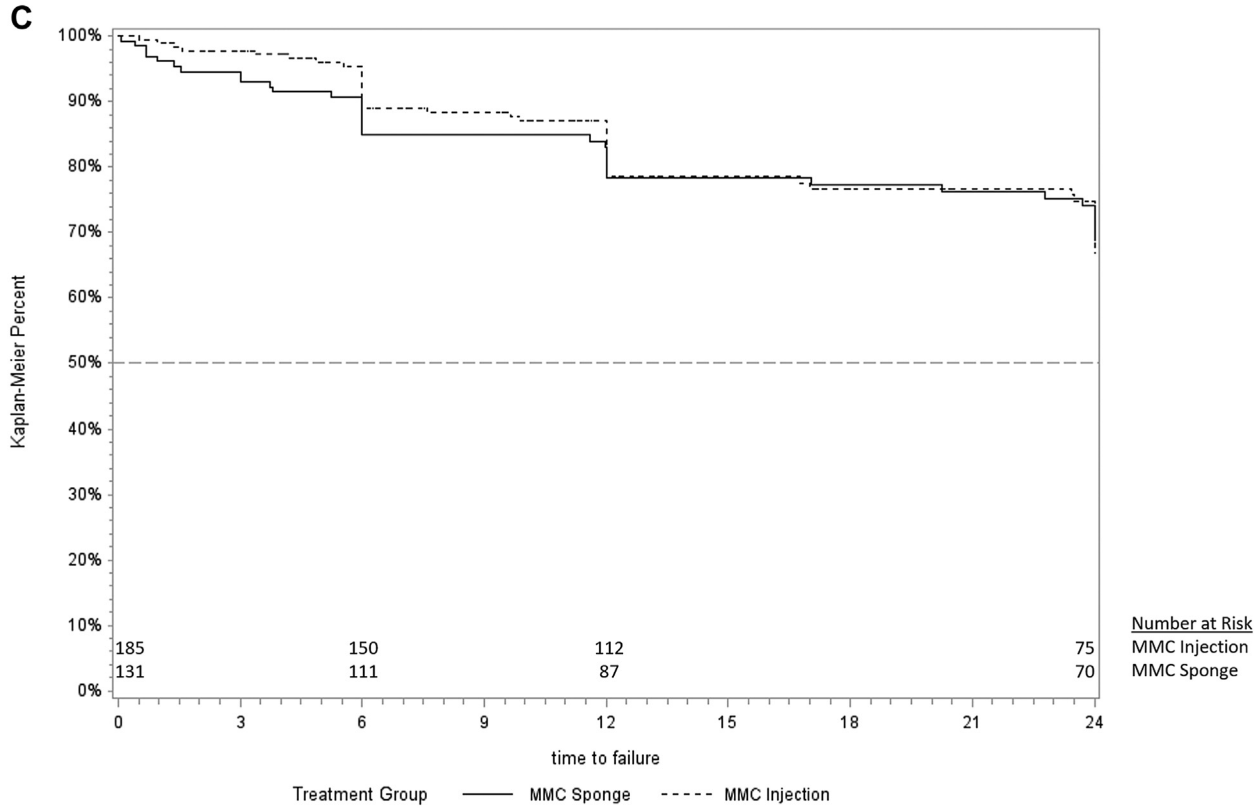

FIGURE 2. *Continued.*

criteria were age less than 18 years, no light perception (NLP) vision, previous incisional eye surgery except for cataract surgery, concurrent surgery in addition to the trabeculectomy, surgery by an early-career surgeon, use of antimetabolite other than MMC, or follow-up less than 1 month. Because this was a retrospective study, the decision to deliver MMC by sponge vs injection was not randomized. However, the choice of MMC delivery was primarily based on different time periods during which delivery of MMC by injection was adopted as a new technique. If the patient received trabeculectomy in more than 1 eye, only the first eye was included in the study. Data collected from the patient medical record included demographic information, ocular diagnoses, prior ocular procedures, ocular measures (visual acuity and IOP measurements pre- and post-surgery, lens status), postoperative complications, and subsequent procedures to control IOP.

• **SURGICAL TECHNIQUE: INJECTION METHOD:** Patients received a retrobulbar block to establish adequate anesthesia and akinesia. After the patient's eye was prepped and draped, a traction suture was placed in the superior cornea and the globe was positioned downwards. The superior conjunctiva was inspected for large conjunctival vessels and ciliary vessels. The antimetabolite (MMC) was injected approximately 8-9 mm posterior to the limbus in an area away from the superior rectus muscle in the sub-Tenon space using a 30 gauge needle. A muscle hook was then used to carefully spread the antimetabolite around Tenon and conjunctiva in the superior region of the globe (Figure 1, A and B). The concentration of MMC used was 0.05-0.4 mg/mL and the total volume delivered was 0.1 mL. The average injected MMC concentration was 0.1 mg/mL. The concentration of MMC was determined by the surgeon based on the characteristics of the individual patient. Next, dissection into the sub-Tenon space was initiated using a limbus-based or a fornix-based conjunctival incision based on surgeon preference and degree of exposure. Once the sub-Tenon space was incised, balanced saline solution on a blunt cannula was used to irrigate the eye. Dissection was continued and trabeculectomy surgery was performed in the conventional manner with a scleral flap made at the 12:00 meridian. A Kelly or Khaw Descemet punch was used to make the ostium, a surgical iridectomy was made, and the scleral flap was closed with interrupted 10-0 nylon suture. The number of sutures placed was left to the discretion of the surgeon. The conjunctiva was closed with either 8-0 or 9-0 Vicryl suture (Johnson & Johnson, New Brunswick, NJ). Dexamethasone was injected into the inferior conjunctival fornix. Atropine and ophthalmic ointment comprising neomycin and polymyxin B sulfates and dexamethasone were placed on the eye prior to patching.

• **SURGICAL TECHNIQUE: SPONGE METHOD:** The surgical steps for this method were the same as for the injection method, with the following differences. When adequate dissection of conjunctiva and Tenon layer was completed, 2-4 cellulose foam sponges that were soaked in MMC were placed underneath conjunctiva and Tenon layer and over the sclera, prior to flap dissection, and left in place for 2-4 minutes, according to the discretion of the surgeon and based on individual patient characteristics. The sponges were then removed and counted. The dose of MMC used was 0.1-0.4 mg/mL, determined by the surgeon based on the characteristics of the individual patient. The site was thoroughly irrigated with balanced saline solution. Trabeculectomy surgery was then performed with the same technique described above.

• **OUTCOME MEASURES:** The primary outcome measure was defined as postoperative mean IOP level. Secondary outcome measures were success and failure rate based on postoperative IOP, glaucoma medication use, complications, early postoperative trabeculectomy interventions (eg, argon laser suture lysis, 5-FU injections), and visual acuity.

Definition of success was categorized as complete or qualified. Complete success was IOP less than 22 mm Hg and IOP reduced by 20% or greater from baseline without the use of glaucoma medications, without additional glaucoma surgery, and without a devastating complication (NLP vision, endophthalmitis). Qualified success was considered the same as above but with the use of glaucoma medications. Kaplan-Meier estimates for the survival of IOP control analyses were performed for IOP less than 22 mm Hg, 18 mm Hg, and 15 mm Hg.

Failure was defined as IOP greater than 21 mm Hg and less than 20% reduction from baseline or IOP less than or equal to 5 mm Hg on 2 consecutive study visits after 3 months, accompanied by loss of 2 lines of Snellen visual acuity attributed to hypotony, reoperation for further IOP lowering (laser or incisional surgery), loss of light perception, or endophthalmitis. Post-trabeculectomy complication data were collected and these were categorized as early (occurring ≤1 month after surgery) and late (occurring >1 month after surgery).

• **STATISTICAL ANALYSIS:** We performed a sample size calculation to detect an IOP difference of 2 mm Hg in change in IOP from baseline between the 2 treatment groups where the standard deviation was 3.4 with 80% power and at a 5% level of significance. The sample size needed was 47 for each group. Because of anticipated loss to long-term follow-up and our intention to test other hypotheses during the study, we obtained a substantially larger initial sample size than was indicated. For our analysis of patient demographic data, where the outcome was categorical, we used contingency tables and Fisher exact test to determine significance. For quantitative outcomes comparisons by categorical group (such as age by treatment group), we used the Kruskal-Wallis test to differentiate between levels. We performed Kaplan-Meier analysis to assess time to failure for each treatment group. When analyzing time to failure with multiple explanatory variables,[28] we used an accelerated failure time model procedure (proc lifereg in SAS; SAS Institute, Cary, North Carolina, USA). All analyses were performed using SAS v. 9.4 (SAS Institute).

RESULTS

FIVE-HUNDRED SIXTY-SIX EYES WERE IDENTIFIED AS HAVING undergone trabeculectomy with antimetabolite in an academic eye center over a 13-year study period. Of these eyes, 150 were excluded for having received an antimetabolite other than MMC, having undergone concurrent cataract surgery, or having less than 1 month of postoperative follow-up. An additional 46 were excluded because they were second eyes of the same study subject, 26 were excluded because they were cases performed by an early-career surgeon, 17 were excluded because the patients were under 18 years of age, 9 had undergone a concomitant surgery or procedure during their trabeculectomy, 1 was excluded for having MMC applied by both sponge and injection method, and 1 had light perception visual acuity. Of the remaining 316 eyes, 131 received trabeculectomy with MMC delivered by sponge and 185 eyes by injection.

Table 1 shows the baseline characteristics of the study population. The 2 treatment groups were not significantly different based on demographic data, but the MMC sponge group had a significantly higher proportion of eyes receiving laser trabeculoplasty at baseline than the MMC injection group (52 [40%] vs 48 [26%], respectively) ($P = .010$). The MMC sponge group had a higher mean IOP (23.2 ± 8.3 mm Hg) than the MMC injection group (20.7 ± 8.1 mm Hg) at baseline ($P = .010$) and was also using a lower number of glaucoma medications than the MMC injection group (2.5 ± 1 vs 2.8 ± 1, respectively) ($P = .03$). The MMC sponge group had a greater proportion of limbus-based trabeculectomy than the MMC injection group (102 [78%] vs 91 [50%], respectively) ($P < .0001$), and a significant difference existed between surgeons in the proportion of each type of MMC delivery technique performed. Surgeon 1 delivered MMC by injection in a greater number of eyes in comparison to Surgeon 2 (131 [71%] vs 54 [30%], respectively) ($P = .001$, Table 1). The time period over which MMC was delivered by sponge ranged from 2001 to 2012, with 1 such procedure performed in 2016. The time period for MMC delivered by injection ranged from 2007 to 2016.

• **INTRAOCULAR PRESSURE AND MEDICATIONS:** At all postoperative time points, the mean IOP was lower in

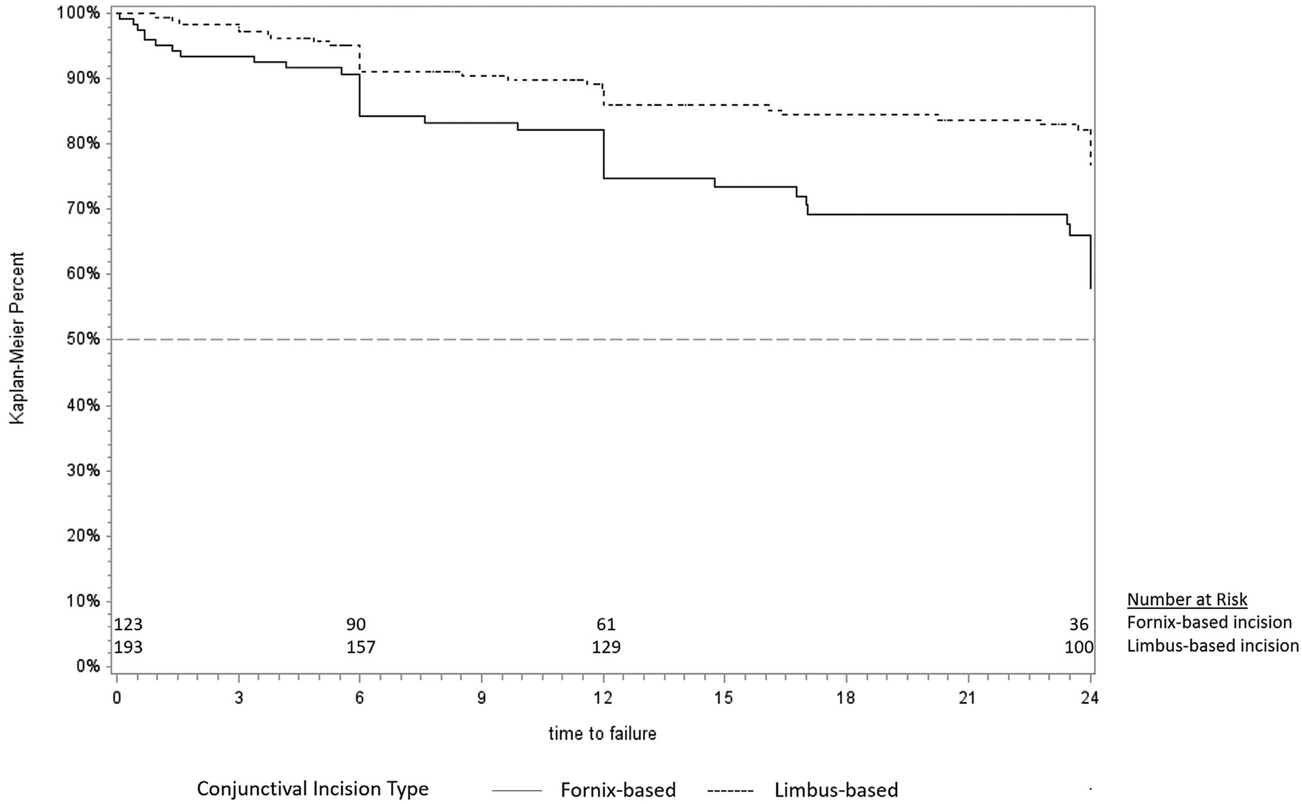

FIGURE 3. Kaplan-Meier estimates for the survival times of intraocular pressure (IOP) control (with or without IOP-lowering medications) for different conjunctival incision types (fornix-based vs limbus-based). Success was defined as IOP $<$22 mm Hg and $>$5 mm Hg and IOP reduced by 20% or greater from baseline without additional glaucoma surgery and without a devastating complication (no light perception vision, endophthalmitis).

TABLE 3. Reasons for Failure in Each Mitomycin-C Delivery Group

Reasons for Failure	MMC Sponge Group (Total Failures = 33) N	%	MMC Injection Group (Total Failures = 39) N	%	*P* Value
IOP >21 mm Hg or not reduced by 20%	10	30	20	51	.09
Reoperation for uncontrolled glaucoma	21	64	14	36	
IOP ≤5 mm Hg AND Snellen visual acuity loss ≥2 lines	2	6	4	10	
No light perception vision	0	0	1	3	

IOP = intraocular pressure; MMC = mitomycin-C.

each of the treatment groups, but no significant difference was noticed between the groups (Table 2). However, as mentioned previously, the IOP was lower in the MMC injection group at baseline prior to trabeculectomy. Therefore, the IOP change from baseline between groups was compared (Table 2) and the MMC sponge group had a slightly greater decrease in IOP from baseline at postoperative month 24, which was significant ($P = .038$). The MMC sponge group required significantly fewer medications to control IOP at the 6-month postoperative time point ($P = .044$) but not at any other postoperative time point (Table 2).

• **PROBABILITY-OF-SURVIVAL AND TIME-TO-FAILURE OUTCOMES:** Kaplan-Meier estimates for the survival times of IOP control (with or without IOP-lowering medications) were not significantly different between MMC treatment groups using different IOP threshold criteria

TABLE 4. Early and Late Post-trabeculectomy Complications

Complication	Early Postoperative Complications (≤1 Month)					Late Postoperative Complications (>1 Month)				
	MMC Sponge Group (N = 131)		MMC Injection Group (N = 185)		*P* Value	MMC Sponge Group (N = 131)		MMC Injection Group (N = 185)		*P* Value
Hyphema	5	4%	11	6%	.45	1	0.8%	1	0.5%	1.00
Choroidal hemorrhage	1	0.8%	3	2%	.65	0	0%	0	0%	N/A
Cataract formation after surgery	1	0.8%	1	0.6%	1.00	19	14%	19	10%	.29
Choroidal effusions	8	6 %	17	9%	.40	0	0%	5	3%	.08
Tense, vascularized, or encapsulated bleb	12	9%	27	15%	.17	12	9%	6	3%	.046
Hypotony/maculopathy	0	0%	6	3%	.044	3	2%	5	3%	1.00
Blebitis	1	0.8%	0	0%	.42	1	0.8%	2	1%	1.00
Cystoid macular edema	0	0%	1	0.5%	1.00	1	0.8%	1	0.5%	1.00
Bleb leak	7	5%	15	8%	.38	7	5%	7	4%	.58
Overfiltration	4	3%	8	4%	.77	4	0%	2	1%	.24
Ostium obstructed by blood or iris	3	2%	3	2%	.70	2	2%	1	0.5%	.57
Iritis	0	0%	0	0%	N/A	2	2%	3	2%	1.00
Corneal decompensation	0	0%	0	0%	N/A	0	0%	1	0.54%	1.000
Bleb dysesthesia	0	0%	0	0%	N/A	0	0%	6	3.24%	.044
Malignant glaucoma	1	0.76%	0	0%	.415	0	0%	1	0.54%	1.000

MMC = mitomycin-C; N/A = not applicable.

(Figure 2, A-C). The proportion of eyes achieving IOP <22 mm Hg at 24 months was 69.7% for the MMC sponge group and 70.5% for the MMC injection group ($P = .65$). The proportion of eyes achieving IOP <18 mm Hg at 24 months was 68.7% for the MMC sponge group and 69.3% for the MMC injection group ($P = .72$). The proportion of eyes achieving IOP <15 mm Hg at 24 months was 68.9% for the MMC sponge group and 66.8% for the MMC injection group ($P = .96$). Using the definition of complete success (no IOP-lowering medications), no significant difference existed for the probability of survival of IOP control between groups at any study time point at any IOP less than 22 mm Hg, 18 mm Hg, or 15 mm Hg.

We have noted a few areas in which significant differences exist between baseline characteristics of the 2 treatment groups. We therefore present a more complex model (accelerated failure time model procedure)[28] to account for these variables. We modeled the log of the time to failure for IOP control, accounting for censoring, against the following explanatory variables: treatment group, glaucoma type, lens type, incision type, surgeon, age at date of surgery, total number of prior glaucoma lasers, preoperative visual acuity (logMAR), and number of glaucoma medications. The first 5 variables are treated as categorical factors and the rest are quantitative covariates. In this model, significant differences were found in only 2 of the variables. Limbus-based conjunctival incision type had a longer time to failure than fornix-based incisions ($P = .001$) and patients of older age had a significantly longer time to failure for IOP control ($P = .0027$). Figure 3 shows the Kaplan-Meier estimates for the survival times of IOP control (for IOP <21 mm Hg and 20% lowering from baseline, with or without IOP-lowering medications) for each conjunctival incision type in order to illustrate the difference between groups. Notably, treatment type (MMC sponge vs MMC injection), which was the primary variable of interest in this study, was not statistically significantly related to time to failure for IOP control ($P = .111$).

• **REASONS FOR FAILURE, COMPLICATIONS, OFFICE PROCEDURE INTERVENTIONS:** The reasons for failure (Table 3) were analyzed and no significant difference was noted between treatment groups ($P = .09$). However, the MMC injection group had a higher proportion of eyes that failed due to IOP greater than 21 mm Hg or less than 20% reduction from baseline and the MMC sponge group had a higher proportion of eyes that failed due to reoperation for uncontrolled glaucoma.

Table 4 shows early (≤1 month) and late (>1 month) complications; for both time periods, the proportion of patients suffering a complication in each treatment group was not significantly different (early: MMC sponge 36/131 [27.5%], MMC injection 69/185 [37.3%] subjects, $P = .07$; late: MMC sponge 48/131 [36.6%], MMC injection 59/185 [31.9%] subjects, $P = .40$). For early complications, the MMC injection group had a significantly greater proportion of cases with hypotony maculopathy, although no significant difference existed between treatment groups

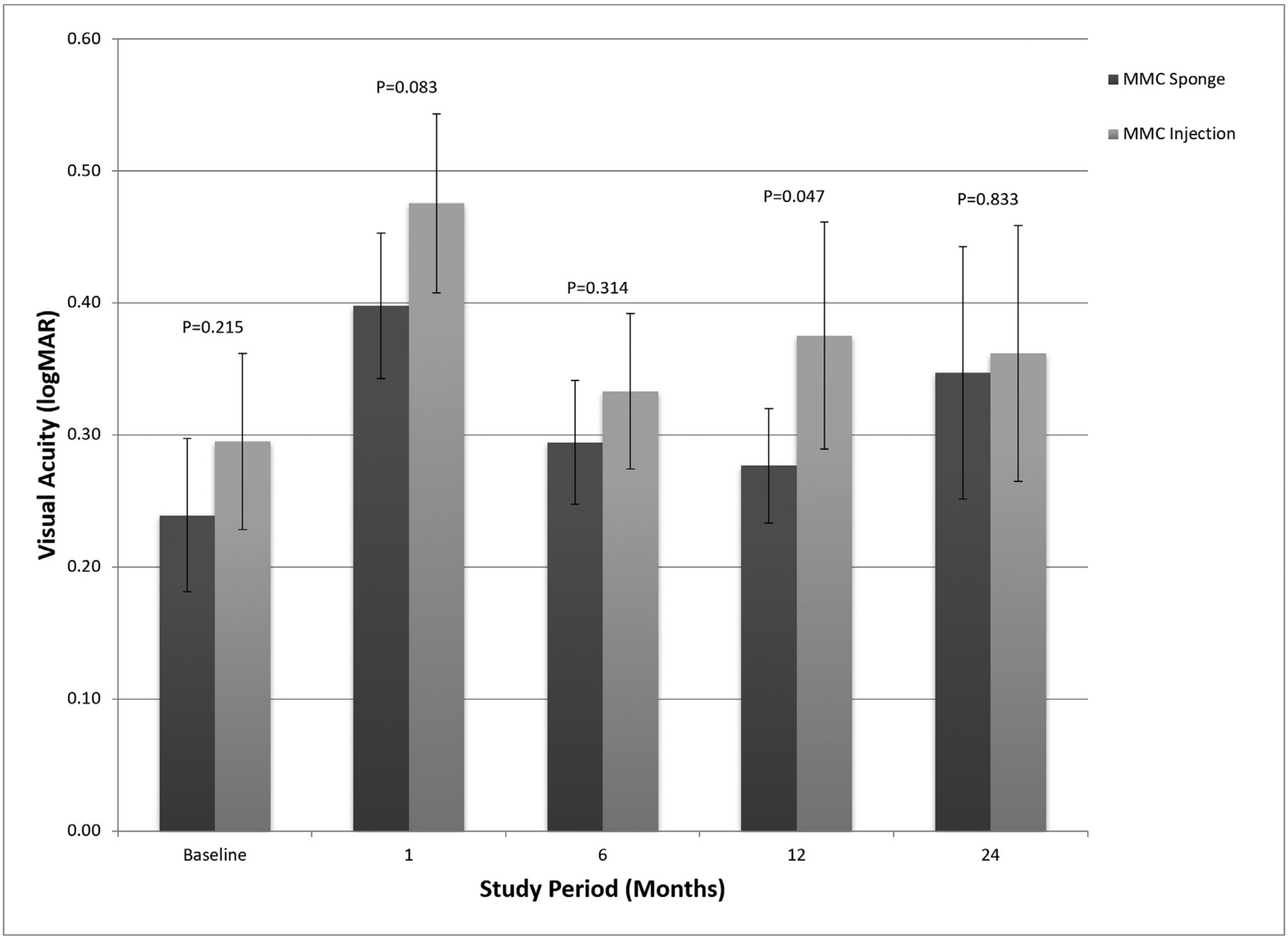

FIGURE 4. Visual acuity measured in logMAR units at baseline and after trabeculectomy surgery in eyes receiving mitomycin-C (MMC) by sponge application and by intra-Tenon injection.

after postoperative month 1 for this particular complication. For late complications, the MMC sponge group had a significantly greater proportion of cases with tense, vascularized, or encapsulated blebs and the MMC injection group had a significantly greater proportion of cases with bleb dysesthesia (Table 4).

Postoperative office procedures were compared between MMC sponge and MMC injection groups and no differences existed for the following procedures (respectively): bleb needling (5/131 [3.8%] vs 10/185 [5.4%], $P = .60$), postoperative 5-FU injection (23/131 [17.6%] vs 36/185 [19.5%], $P = .77$), trabeculectomy flap suture lysis (77/131 [58.7%] vs 109/185 [58.9%], $P = 1.00$), and viscoelastic reformation of the anterior chamber (4/131 [3.05%] vs 1/185 [0.54%], $P = .16$).

• **VISUAL ACUITY:** The difference in visual acuity between treatment groups was not significantly different except at postoperative month 12, when the MMC sponge group had a slightly better mean logMAR visual acuity measure in comparison to MMC injection ($P = .047$) (Figure 4), but by month 24 the difference in vision between groups was not significant ($P = .83$). The difference in logMAR visual acuity between each postoperative visit and baseline was not significantly different between treatment groups. The percentage of subjects who lost 2 or more lines of Snellen visual acuity was calculated for each study time point and the differences between each treatment group were analyzed. For MMC sponge and MMC injection groups, respectively, at 6 months 35 of 110 (31.8%) and 36 of 151 (23.8%) subjects ($P = .16$), at 1 year 27 of 94 (28.7%) and 28 of 124 (22.6%) subjects ($P = .35$), and at 2 years 27 of 84 (32.1%) and 26 of 92 (28.3%) subjects ($P = .62$) lost 2 lines or more of Snellen visual acuity.

DISCUSSION

THIS RETROSPECTIVE, SINGLE-CENTER STUDY COMPARES the outcomes of trabeculectomy surgery with MMC applied by intra-Tenon injection vs sponge. We found that both techniques could effectively lower IOP in patients with glaucoma and that the rate of success for IOP control was not significantly different between MMC sponge and injection technique at any time point. We did find that the change in IOP from baseline was significantly lower in the MMC sponge group than in the MMC injection group at postoperative month 24. At this same time point, no significant differences existed between groups for mean medication use. An analysis examining log of the time to failure for IOP control showed no differences between MMC treatment groups. However, greater patient age and limbus-based conjunctival incision were associated with longer time to failure of IOP control.

In our study, we hypothesized that a diffuse application of MMC would offer an advantage in lowering IOP by increasing the surface area of the conjunctiva and Tenon layer that was treated, but instead we found that both mean postoperative IOP and success rate for IOP control were not different between groups. The finding that the MMC sponge group had a greater decrease in IOP from baseline at month 24 could be interpreted in 2 ways: either MMC delivered by sponge is more effective in lowering IOP or the lower preoperative IOP in the MMC injection group made it more difficult to exert a change. Other studies of MMC injection or irrigation with trabeculectomy surgery in human eyes likewise show similar mean IOP outcomes in comparison to sponge groups as well as no statistically significant difference in success rate of IOP control.[21,23,27] However, 1 study did find a significantly greater change in IOP from baseline in the MMC irrigation group at postoperative month 6 in comparison to the MMC sponge group in eyes treated with trabeculectomy and EX-PRESS shunt.[23]

Our study revealed a surprising finding in that trabeculectomy surgeries performed with limbus-based conjunctival incisions had a longer time to failure for IOP control. The literature regarding the effect of conjunctival incision type on post-trabeculectomy IOP outcomes is mixed.[29–31] Some studies have shown no difference in IOP outcomes when comparing fornix-based and limbus-based trabeculectomy. For example, Solus and associates[29] analyzed 652 eyes undergoing trabeculectomy surgery and found that the success rate for IOP control was no different between conjunctival incision types. In another study, Al-Haddad and associates[31] performed a meta-analysis and no difference in effectiveness for IOP control was found between fornix-based vs limbus-based trabeculectomy surgery, although the authors point out that the findings were uncertain owing to a "small number of events" gleaned from multiple publications. However, other studies report different outcomes; for example, Hirayama[32] found a lower postoperative IOP in the limbus-based group and Fukuchi and associates[30] found more frequent failure in the fornix-based group during the "early postoperative period." In addition, Fontana and associates[33] studied the outcomes of trabeculectomy performed with MMC in pseudophakic eyes with open-angle glaucoma and found a lower risk of IOP failure in eyes with limbus-based conjunctival incisions (hazard ratio, 0.32; 95% CI, 0.1-0.8; $P = .012$). A proposed explanation for our finding is that fornix-based trabeculectomy may have higher risk for early leak or wound dehiscence, which could potentially compromise success of IOP control in the long term, and the study by Solus and associates[29] did report a higher rate of symptomatic hypotony in their fornix-based group. The compromise of success may occur because bleb massage and trabeculectomy flap suture lysis (by laser) may not be possible when a leak is present, and these are maneuvers glaucoma surgeons perform to encourage filtration in the early postoperative period.

Our finding that older patient age was associated with longer time to fail for IOP control is consistent with outcomes reported in other studies. For example, Gressel and associates[34] reported trabeculectomy outcomes in patients of young age at a time when MMC was not used. In this study, a higher success rate was noted in the older age bracket. Of 45 trabeculectomies performed on patients aged 10-29 years, 17 (38%) were successful; and of 66 trabeculectomies performed on patients aged 30-49 years, 43 (65%) were successful. The Advanced Glaucoma Intervention Study (AGIS) found that older age was associated with a lower risk of trabeculectomy failure.[35] For trabeculectomy as a first or second intervention, the risk of failure decreased by 3% for every 1-year increase in preintervention age (HR: 0.97; CI 0.95-0.99; $P = .005$). Of note, this study took place over a long period of time (1988 to 2001), during which the use of antimetabolites evolved. Thus, trabeculectomies in this study were performed with either no antimetabolite, 5-FU, or MMC. Fontana and associates[33] found that increasing age was associated with a lower risk of failure to control IOP (hazard ratio, 0.90; 95% CI, 0.8-1.0 for success in IOP control <15 mm Hg and $>25\%$ pressure reduction or a reduction of at least 2 medications ($P = .07$) and <12 mm Hg and $>30\%$ pressure reduction or a reduction of at least 2 medications ($P = .027$)).

Postoperative complications reported in this study were similar between treatment groups, with a few exceptions. A greater rate of hypotony maculopathy was noted in the MMC injection group early but not late. Quist and associates[23] found a higher proportion of hypotony in the MMC irrigation group but not hypotony maculopathy, while Pakravan and associates[21] and Khouri and associates[27] found no difference for this particular complication. Among 4 recent publications comparing MMC delivered by sponge vs injection or irrigation, no differences in complications were found for bleb leak, choroidal effusions, suprachoroidal hemorrhage, overfiltration, or infection.[21–23,27] Only 1 study to date has investigated endothelial cell count (ECC) before and after trabeculectomy surgery with MMC delivered by sponge vs injection. In this prospective, randomized investigation, eyes randomized to MMC injection had a preoperative ECC of 2843 $\pm$ 391 and eyes randomized to MMC delivered by sponge had an ECC of 2875 $\pm$ 374 ($P = .858$).[21] Postoperatively, the ECC did not seem to change within groups or between groups (MMC injection 2843 ± 387 and MMC sponge 2877 ± 376, $P = .813$), although this was a short, 6-month study.

The MMC sponge group had significantly more tense/vascularized/encapsulated blebs as a late complication than the MMC injection group. The proportion of this type of bleb found in the MMC sponge group was in a range similar to that found in the Tube Versus Trabeculectomy Study (6%), a major prospective clinical trial involving trabeculectomy in which MMC was delivered by the traditional sponge method.[24] Pakravan and associates[21] noted a higher bleb vascularization score in eyes with MMC delivered by sponge at postoperative month 6, whereas 1 other study[23] did not find a difference in bleb vascularity grading. Two studies comparing MMC delivered by sponge vs irrigation found a significantly higher frequency of 5-FU injections in the MMC sponge group.[23,27] These findings provide some evidence that injected MMC may offer a benefit in reducing the degree of conjunctival wound healing or scarring in the long-term postoperative period, although one should keep in mind that surgeons were not masked to MMC delivery technique and the aggressiveness of their postoperative management could be influenced by this. Another clue to bleb morphology in our study was that the MMC injection subjects had a significantly greater proportion of eyes with bleb dysesthesia and this may suggest that these blebs are higher or perhaps more diffuse.

The safety of injecting MMC into conjunctival and Tenon tissue has been studied in animals and the concentrations of this drug in ocular tissues after injection have been determined.[19,20] In rabbits, MMC injected into the subconjunctival space resulted in ocular tissue concentrations that were slightly higher than MMC delivered by sponge[20]; however, the levels of MMC in conjunctiva, sclera, and aqueous humor were still generally low and became undetectable after 24-72 hours.[19] The rapid decline in MMC levels in ocular tissues suggests that injecting this drug before a fistula to the anterior chamber has been created is no more toxic than delivering it by sponge, and our study in human eyes provides some evidence for this.

At the 12-month postoperative time point, the MMC sponge group had statistically significantly better vision as measured by logMAR units, but at all other time points the difference was not significant between groups. Two other studies comparing MMC injection or irrigation report no difference in visual acuity between groups[23,27] and no difference in change in visual acuity within each treatment group,[23] which further supports the safety of this delivery technique.

The reasons for failure among our treatment groups revealed that when failure occurred, the MMC sponge group was more likely to fail by need for glaucoma reoperation, whereas the MMC injection group was more likely to fail by IOP greater than 21 mm Hg or less than 20% reduction from baseline. These findings imply that when failure occurred, the MMC injection group may have been controlled by escalating IOP-lowering medication and that further glaucoma surgery could be avoided. Other studies corroborate our findings, and in 1 prior prospective study comparing MMC applied by sponge vs irrigation,[23] the sponge group had a higher rate (4/44; 9%) of glaucoma reoperation than the incision group. Likewise, in a small retrospective study comparing MMC sponge vs MMC irrigation,[27] 3 of 30 (10%) subjects in the former group required further glaucoma surgery, vs 1 of 30 (3.3%) in the latter group.

Three advantages of MMC injection that were not specifically investigated in this study were reduction in MMC application time, elimination of the risk of lost or retained sponge material, and the delivery of a known amount of MMC. The average application time of MMC by sponge is 3-5 minutes, whereas the injection method takes approximately 30 seconds to perform. Retained MMC sponges during trabeculectomy surgery have been a point of consternation among glaucoma surgeons and can cause tissue necrosis if not recognized quickly.[36] With the injection technique, this rare but worrisome surgical complication is eliminated. MMC delivery by injection offers the advantage of delivering a known amount of medication to the surgical site, since the concentration of prepared drug and the volume injected can be used to calculate the exact amount of drug delivered to the target tissues. This ability to calculate an exact dose lends itself well to prospective research studies of glaucoma procedures requiring MMC, such as new minimally invasive glaucoma procedures that shunt aqueous humor to the subconjunctival space. With MMC delivered by sponge, the actual dose of medication that is transferred from sponge to ocular tissues is unknown.

Our study has several limitations, most important of which is that it is a nonrandomized, retrospective study. In our study, MMC (drug dose in the case of injection application, drug concentration, and duration in sponge application) was not randomized and was subject to surgeons' preferences. Similarly, the conjunctival incision technique (limbus- or fornix-based) was not randomly assigned and it was subject to surgeon preference. At baseline, the MMC sponge group had a higher proportion of limbus-based conjunctival incisions and the MMC injection group had a higher proportion of fornix-based incisions. Although we found that conjunctival incision technique did independently influence time to failure for IOP control, it is important to note that MMC application technique, our main study interest, did not.

In summary, our study demonstrates that MMC injection during routine trabeculectomy lowers IOP in a fashion like that of the more traditional method of MMC delivery by sponge and that the safety profile is likewise comparable. A surprising finding was that limbus-based conjunctival incision was associated with a significantly longer time to failure for postoperative IOP control. The advantages of our study are the large sample size of 316 eyes and the long-term follow-up of 24 months. In conclusion, MMC application by injection during trabeculectomy surgery is a viable method of antimetabolite delivery; in addition, it is faster than applying MMC by sponge, the risk of leaving a sponge behind is absent, and the dose of MMC delivered to the ocular tissues is known.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

MICHELE C. LIM: CONCEPTUALIZATION, METHODOLOGY, Investigation, Data curation, Formal analysis, Writing - original draft, Visualization, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Betty Hom:** Investigation, Data curation, Formal analysis, Writing - review & editing, Funding acquisition. **Mitchell R. Watnik:** Conceptualization, Methodology, Formal analysis, Visualization, Writing - review & editing. **James D. Brandt:** Conceptualization, Methodology, Writing - review & editing. **Allison R. Altman:** Investigation, Data curation, Writing - review & editing. **Tania Paul:** Conceptualization, Methodology, Investigation, Data curation, Writing - review & editing. **Melissa G. Tong:** Conceptualization, Methodology, Investigation, Data curation, Writing - review & editing.

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REFERENCES

1. Cairns JE. Trabeculectomy. Preliminary report of a new method. *Am J Ophthalmol* 1968;66(4):673–679.
2. Watson PG, Barnett F. Effectiveness of trabeculectomy in glaucoma. *Am J Ophthalmol* 1975;79(5):831–845.
3. Razeghinejad MR, Fudemberg SJ, Spaeth GL. The changing conceptual basis of trabeculectomy: a review of past and current surgical techniques. *Surv Ophthalmol* 2012;57(1):1–25.
4. Lama PJ, Fechtner RD. Antifibrotics and wound healing in glaucoma surgery. *Surv Ophthalmol* 2003;48(3):314–346.
5. Fluorouracil Filtering Surgery Study one-year follow-up. The Fluorouracil Filtering Surgery Study Group. *Am J Ophthalmol* 1989;108(6):625–635.
6. Hata T, Hoshi T, Kanamori K, et al. Mitomycin, a new antibiotic from Streptomyces. I. *J Antibiot (Tokyo)* 1956;9(4): 141–146.
7. Kim K-W, Roh JK, Wee H-J, Qn G. Cancer drug discovery: science and history. Dordrecht: Springer; 2016.
8. Seibold LK, Sherwood MB, Kahook MY. Wound modulation after filtration surgery. *Surv Ophthalmol* 2012;57(6):530–550.
9. Chen CW, Huang HT, Bair JS, Lee CC. Trabeculectomy with simultaneous topical application of mitomycin-C in refractory glaucoma. *J Ocul Pharmacol* 1990;6(3):175–182.
10. Vinod K, Gedde SJ, Feuer WJ, et al. Practice preferences for glaucoma surgery: a survey of the American Glaucoma Society. *J Glaucoma* 2017;26(8):687–693.
11. Wells AP, Cordeiro MF, Bunce C, Khaw PT. Cystic bleb formation and related complications in limbus- versus fornix-based conjunctival flaps in pediatric and young adult trabeculectomy with mitomycin C. *Ophthalmology* 2003;110(11): 2192–2197.
12. Yamamoto T, Sawada A, Mayama C, et al. The 5-year incidence of bleb-related infection and its risk factors after filtering surgeries with adjunctive mitomycin C: collaborative bleb-related infection incidence and treatment study 2. *Ophthalmology* 2014;121(5):1001–1006.
13. Soltau JB, Rothman RF, Budenz DL, et al. Risk factors for glaucoma filtering bleb infections. *Arch Ophthalmol* 2000; 118(3):338–342.
14. Drolsum L, Willoch C, Nicolaissen B. Use of amniotic membrane as an adjuvant in refractory glaucoma. *Acta Ophthalmol Scand* 2006;84(6):786–789.
15. You YA, Gu YS, Fang CT, Ma XQ. Long-term effects of simultaneous subconjunctival and subscleral mitomycin C application in repeat trabeculectomy. *J Glaucoma* 2002; 11(2):110–118.
16. Bruno CA, Eisengart JA, Radenbaugh PA, Moroi SE. Subconjunctival placement of human amniotic membrane during high risk glaucoma filtration surgery. *Ophthalmic Surg Lasers Imaging* 2006;37(3):190–197.
17. Jones E, Clarke J, Khaw PT. Recent advances in trabeculectomy technique. *Curr Opin Ophthalmol* 2005;16(2):107–113.
18. Cordeiro MF, Constable PH, Alexander RA, Bhattacharya SS, Khaw PT. Effect of varying the mitomycin-C treatment area in glaucoma filtration surgery in the rabbit. *Invest Ophthalmol Vis Sci* 1997;38(8): 1639–1646.
19. Kawase K, Matsushita H, Yamamoto T, Kitazawa Y. Mitomycin concentration in rabbit and human ocular tissues after topical administration. *Ophthalmology* 1992;99(2):203–207.
20. Mietz H, Diestelhorst M, Rump AF, Theisohn M, Klaus W, Krieglstein GK. Ocular concentrations of mitomycin C using different delivery devices. *Ophthalmologica* 1998;212(1): 37–42.
21. Pakravan M, Esfandiari H, Yazdani S, et al. Mitomycin C-augmented trabeculectomy: subtenon injection versus soaked sponges: a randomised clinical trial. *Br J Ophthalmol* 2017; 101(9):1275–1280.
22. Lee E, Doyle E, Jenkins C. Trabeculectomy surgery augmented with intra-Tenon injection of mitomycin C. *Acta Ophthalmol* 2008;86(8):866–870.
23. Quist MS, Brown N, Bicket AK, Herndon LW. The short-term effect of subtenon sponge application versus subtenon

irrigation of mitomycin-C on the outcomes of trabeculectomy with Ex-PRESS glaucoma filtration device: a randomized trial. *J Glaucoma* 2018;27(2):148–156.
24. Gedde SJ, Herndon LW, Brandt JD, Budenz DL, Feuer WJ, Schiffman JC. Surgical complications in the Tube Versus Trabeculectomy Study during the first year of follow-up. *Am J Ophthalmol* 2007;143(1):23–31.
25. Lichter PR, Musch DC, Gillespie BW, et al. Interim clinical outcomes in the Collaborative Initial Glaucoma Treatment Study comparing initial treatment randomized to medications or surgery. *Ophthalmology* 2001;108(11):1943–1953.
26. Gedde SJ, Schiffman JC, Feuer WJ, Herndon LW, Brandt JD, Budenz DL. Three-year follow-up of the Tube Versus Trabeculectomy Study. *Am J Ophthalmol* 2009;148(5):670–684.
27. Khouri AS, Huang G, Huang LY. Intraoperative injection vs sponge-applied mitomycin C during trabeculectomy: one-year study. *J Curr Glaucoma Pract* 2017;11:101–106.
28. Altman DG, Gore SM, Gardner MJ, Pocock SJ. Statistical guidelines for contributors to medical journals. *Br Med J (Clin Res Ed)* 1983;286(6376):1489–1493.
29. Solus JF, Jampel HD, Tracey PA, et al. Comparison of limbus-based and fornix-based trabeculectomy: success, bleb-related complications, and bleb morphology. *Ophthalmology* 2012;119(4):703–711.
30. Fukuchi T, Ueda J, Yaoeda K, Suda K, Seki M, Abe H. Comparison of fornix- and limbus-based conjunctival flaps in mitomycin C trabeculectomy with laser suture lysis in Japanese glaucoma patients. *Japan J Ophthalmol* 2006;50(4):338–344.
31. Al-Haddad CE, Abdulaal M, Al-Moujahed A, Ervin A-M, Ismail K. Fornix-based versus limbal-based conjunctival trabeculectomy flaps for glaucoma: findings from a Cochrane Systematic Review. *Am J Ophthalmol* 2017;174:33–41.
32. Hirayama S. Limbus-based vs. fornix-based conjunctival flaps in trabeculectomy in fellow eyes. *J Eye* 2003;20:999–1003.
33. Fontana H, Nouri-Mahdavi K, Caprioli J. Trabeculectomy with mitomycin C in pseudophakic patients with open-angle glaucoma: outcomes and risk factors for failure. *Am J Ophthalmol* 2006;141(4):652–659.
34. Gressel MG, Heuer DK, Parrish RK 2nd. Trabeculectomy in young patients. *Ophthalmology* 1984;91(10):1242–1246.
35. The Advanced Glaucoma Intervention Study (AGIS). 11. Risk factors for failure of trabeculectomy and argon laser trabeculoplasty. *Am J Ophthalmol* 2002;134(4):481–498.
36. Shin DH, Tsai CS, Kupin TH, Olivier MM. Retained cellulose sponge after trabeculectomy with adjunctive subconjunctival mitomycin C. *Am J Ophthalmol* 1994;118(1):111–112.