

Limbal Stem Cell Deficiency after Topical Mitomycin C Therapy for Primary Acquired Melanosis with Atypia

Alejandro Lichtinger, MD, Jacob Pe'er, MD, Joseph Frucht-Pery, MD, Abraham Solomon, MD

Purpose: To describe the incidence, characteristics, risk factors, and clinical outcome of limbal stem cell deficiency (LSCD) resulting from topical treatment with mitomycin C (MMC) for primary acquired melanosis (PAM) with atypia.

Design: Retrospective, observational case series.

Participants: Patients with LSCD who had been managed with topical MMC for PAM with atypia at the Ocular Oncology Service at the Department of Ophthalmology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel, between 2000 and 2007.

Methods: Retrospective chart review of all patients with PAM with atypia was performed. Impression cytologic analysis of the corneal and conjunctival epithelium was performed in patients suspected of having LSCD.

Main Outcome Measures: Evaluation of risk factors for LSCD, including demographic characteristics, MMC dosage, and length of treatment; and clinical and visual outcome of patients diagnosed with LSCD.

Results: Limbal stem cell deficiency was identified in 5 (23.8%) of 21 patients. The mean age \pm standard deviation of the 5 patients was 61.8 ± 12.7 years compared with 43.7 ± 16.1 years in patients in whom this complication did not develop ($P = 0.025$). Longer treatment periods of MMC were noted in eyes in which LSCD developed (78.4 ± 24.8 days) compared with eyes without LSCD (37.7 ± 3.1 days; $P = 0.07$). In 3 patients, spontaneous partial resolution of the LSCD was noted.

Conclusions: High-dose topical MMC for PAM with atypia may be associated with a relatively high incidence of LSCD. Mitomycin C concentration and treatment regimen should be reevaluated to improve the safety of this treatment protocol.

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Primary acquired melanosis (PAM) with atypia carries an almost 50% risk of transformation into malignant melanoma.¹ Surgical excision, cryotherapy, and laser therapy have been reported as possible treatment options. However, these procedures are associated with significant recurrence rates and with many complications.^{2–4}

Topical chemotherapy currently is the leading therapy for the management of ocular surface neoplasia. Topical chemotherapy of conjunctival tumors offers a nonsurgical treatment with less dependence on free surgical margins.^{5,6} It can be used to treat tumor extensions onto the corneal epithelium and easily can be repeated. This approach also allows for a high concentration of chemotherapy to be delivered directly to the tumor.⁷

The use of topical mitomycin C (MMC) for the treatment of ocular surface neoplasia (OSN), including conjunctival-corneal intraepithelial neoplasia (CCIN),^{8–11} PAM with atypia,^{5,12–14} and conjunctival malignant melanoma^{7,13} has been well described since 1994, when it was first reported for CCIN at the authors' institute.¹⁵ Mitomycin C is considered a safe chemotherapeutic agent in treating ocular surface neoplasia. Transient side effects, including tearing, ocular pain, blepharospasm, keratoconjunctivitis,

conjunctival hyperemia, allergic reaction, and punctal stenosis, are common.^{7,9,11,15–18} No long-term complications were recognized so far with topical MMC, and specifically, limbal stem cell deficiency (LSCD) was not reported as a major long-term adverse effect. To the authors' knowledge, there is only one previous case report¹⁹ of LSCD after topical MMC treatment for OSN in a case of CCIN. This retrospective study reports on a relatively high incidence of LSCD after topical MMC therapy in patients who had PAM with atypia.

Patients and Methods

This study included all patients with the diagnosis of PAM with atypia, with a histopathologic confirmation, seen at the Ocular Oncology Service at the Department of Ophthalmology, Hadassah-Hebrew University Medical Center, between 2000 and 2007. The clinical charts of all patients with that diagnosis were retrieved from the Ocular Oncology Service database and were reviewed retrospectively. This study was recognized by the Hadassah-Hebrew University Medical Center Institutional Review Board as a retrospective study and received exemption from the usual review process (file no. 205-05.09.08/2959).

Clinical Management of Patients with Primary Acquired Melanosis with Atypia

The routine protocol for patients with a suspicious pigmented conjunctival lesion at presentation usually includes referral to either the Cornea Service or to the Ocular Oncology Service at the Department of Ophthalmology. An initial evaluation included complete eye examination, and slit-lamp photography was performed. An incisional or excisional biopsy for histopathologic diagnosis was performed in all patients, depending on the size, extent, and location of the lesion. As soon as the diagnosis was confirmed by biopsy and the ocular surface had healed from the surgical procedure, a decision for further treatment with topical chemotherapy was made, based on type of biopsy, surgical margins, and extent of the lesion.

In patients treated with topical chemotherapy, the protocol consisted of at least 3 cycles of topical 0.02% or 0.04% MMC, 4 times daily for 14 consecutive days, with an interval of 2 weeks between cycles. During the interval, patients received topical fluorometholone twice daily and preservative-free tear supplements as needed.

Limbal stem cell deficiency was defined as the appearance of abnormal and irregular corneal epithelium, with an increased fluorescein staining resembling a conjunctival staining pattern, and the appearance of a least 1 goblet cell in the corneal surface on impression cytologic specimens. The suspicion of LSCD was based clinically on the appearance of the corneal epithelium, which presented with hazy, irregular, and opacified epithelium; recurrent corneal erosions; and a decrease in the best-corrected visual acuity. The location of the abnormal epithelium varied among patients: in some of the patients, the epithelial involvement indeed was noted at the periphery of the cornea at the beginning and later extended to the center, whereas in others, it was noted as involving the entire cornea. Identified cases of suspected LSCD underwent full ophthalmologic examination and clinical slit-lamp photography, including blue-filtered photographs with topical fluorescein. In addition, impression cytologic analysis was performed to confirm the diagnosis of LSCD.

Impression Cytologic Analysis

Impression cytologic analysis was performed on suspicion of LSCD, as previously described by Prabhasawat and Tseng²⁰ and by Tseng.²¹ Briefly, nitrocellulose filter paper (Milipore, Bedford, MA) with a pore size of 0.22 mm was cut into asymmetrical rectangular pieces. A piece of this filter paper was applied over the limbal area after application of a drop of topical anesthetic. Four pieces of filter paper were applied to each patient—each over 1 quadrant, respectively. Each piece covered an area of limbus including the adjacent cornea and conjunctiva. The filter paper pieces were pressed gently against the ocular surface for 5 seconds, and then were peeled off and placed immediately in a fixative containing 70% alcohol and 37% ethyl alcohol in glacial acetic acid.

Specimens were stained with periodic acid–Schiff–Gill's modified Papanicolaou stain. Specimens were rehydrated in consecutive decreasing concentrations of ethanol, and then were oxidized for 5 minutes in 0.5% periodic acid, rinsed in distilled water, immersed for 5 minutes in pure Schiff reagent, soaked for 2 minutes in 0.5% sodium bisulfite, and soaked in running tap water for an additional 5 minutes. Specimens then were counter-stained with Gill's hematoxylin for 2 minutes, followed by 3 minutes' immersion in 0.5% hydrochloride solution, and afterward were rinsed in tap water. After dehydration in 95% ethanol, specimens were stained with orange G6 for 2 minutes and with EA 50 dye, followed by destaining in 95% ethanol after each dye. Specimens

then were dehydrated in absolute alcohol, immersed in xylene, and mounted on cover-slipped slides.

The cytologic evidence of LSCD primarily is based on the presence of goblet cell-containing conjunctival epithelial cells that migrate to the cornea, a process termed *conjunctivalization*.²² Therefore, a diagnosis of LSCD was made based on the appearance of goblet cells on the areas corresponding to the corneal surface and on the appearance of migrating epithelial cells from the conjunctival to the corneal surface.

Management of Limbal Stem Cell Deficiency

All patients with LSCD were followed-up on a monthly basis. Patients received topical preservative-free tear substitutes and were maintained on topical fluorometholone to suppress signs of ocular surface inflammation. Recurrent corneal erosions were managed with an extended wear soft bandage contact lens, which was applied for up to 2 weeks.

Statistical Analysis

Univariate analysis was performed to evaluate possible risk factors for LSCD in patients who received MMC for PAM with atypia.

Results

During the study period, 24 patients with the diagnosis of PAM with atypia were identified. All of them had incisional or excisional biopsy to confirm the diagnosis. In 21 of these patients (7 males and 14 females) topical chemotherapy with MMC was applied as part of their treatment. None of the patients had a history of a previous systemic malignancy, and none had received any systemic chemotherapy treatment before or during the study period. In addition, none of the patients had any previous ocular surface disorder.

In a total of 5 patients (23.8%), 1 male and 4 females, LSCD developed after treatment with MMC (Table 1). All of these patients had an irregular staining pattern of the corneal epithelium (Figs 1B and 2B). Impression cytologic analysis of the limbal area from these patients demonstrated goblet cells in the corneal epithelium (Figs 1C and 2C), and a typical migration pattern of conjunctival epithelial cells into the corneal surface (Fig 2C).

The mean age of patients with LSCD was significantly higher than the age of patients who received MMC but did not have LSCD: 61.8 ± 12.7 years compared with 43.7 ± 16.12 years, respectively ($P = 0.025$).

The best-corrected visual acuity (BCVA) in patients with LSCD at the time of diagnosis of PAM with atypia was 0.84, compared with 0.91 in those in whom LSCD did not develop ($P = 0.1092$). On diagnosis of LSCD, the mean BCVA deteriorated to 0.34, and at last follow-up it improved to 0.54 (compared with 0.78 for patients with no LSCD; $P = 0.05$; Table 2).

The time that elapsed between the last cycle of MMC treatment and the diagnosis of LSCD varied from 0 to 24 months (mean, 7.6 months). The number of cycles of MMC varied from 3 to 12 cycles (mean, 5.6 cycles). Each treatment cycle lasted for 14 consecutive days. Therefore, the total treatment period of topical MMC in patients in whom LSCD developed ranged between 42 and 168 days (Table 1).

The mean total treatment period with MMC in eyes in which LSCD developed was more than twice that for patients in whom LSCD did not develop: 78.4 ± 24.8 days compared with 37.7 ± 3.1 days, respectively ($P = 0.07$, Mann–Whitney U test). Of the 5 patients with LSCD, 2 patients received topical MMC for a total of

Table 1. Demographics, Mitomycin C Dosage, and Clinical Outcome of Patients with Post-Mitomycin C Limbal Stem Cell Deficiency

Case No.	Age (yrs)	Gender	Eye	Mitomycin C Dosage		Cumulative Period Receiving Mitomycin C (Days)	Time from Initial Mitomycin C Course to Limbal Stem Cell Deficiency Diagnosis	Follow-up with Limbal Stem Cell Deficiency (Mos)	Status at Last Visit
				Concentration	Number of Mitomycin C Courses				
1	75	F	Right	0.04%	3	42	During third course	5	Improvement after 2 mos
2	56	M	Right	0.04%	3	42	2 wks after third course	33	Improvement after 31 mos
3	57	F	Right	0.02%	1	42	6 wks after third course	7	Fluctuating vision, LSCD
				0.04%	2				
4	75	F	Left	0.02%	2	98	1 yr after seventh course	65	Fluctuating vision, LSCD
				0.04%	5				
5	46	F	Right	0.02%	2	168	2 yrs after twelfth course	14	Improvement after 10 mos
				0.04%	10				

LSCD = limbal stem cell deficiency.

98 and 168 days, respectively, and 3 patients received MMC for a total of 42 days each (Table 1).

Patients in whom LSCD developed were managed with lubrication and with topical corticosteroids. A soft bandage contact lens was applied when persistent nonhealing epithelial defects were evident. The follow-up from the diagnosis of LSCD was from 5 to 65 months (mean, 24.8 months). The uncorrected visual acuity in these patients had marked fluctuations, associated with variations of ocular surface irregularity and formation of mild corneal haze. In 3 of the patients, spontaneous partial resolution of the LSCD was noted, as normal corneal epithelium seemed gradually to replace the abnormal irregular conjunctival epithelium. This was associated with gradual improvement in the visual acuity.

Case 1

A 75-year-old female was referred to our clinics with a diffuse pigmented lesion involving the bulbar conjunctiva and upper fornix of her right eye (Fig 1A). Migration of pigmented epithelial cells into the cornea was noted. Based on an incisional biopsy, a diagnosis of PAM with atypia was made. The patient was started on topical chemotherapy with MMC.

At diagnosis, her BCVA was 0.8. The patient received 2 courses of topical chemotherapy with 0.04% MMC. During the third course of topical MMC, she reported pain and redness in the right eye, and her BCVA had dropped down to counting fingers at 1 m. On examination, almost 90% of the corneal surface was covered with irregular, opaque epithelium with only a small wedge of healthy epithelium at the temporal limbus (Fig 1B). On impression cytologic analysis, multiple goblet cells were demonstrated in the corneal portion of the specimen, compatible with LSCD (Fig 1C). She was managed with topical corticosteroids and preservative-free tear substitutes. The cornea remained stable, with poor visual acuity for 2 months.

Two months after the initial diagnosis, she experienced mild improvement in her visual acuity, and migration of healthy corneal epithelium was seen from the small temporal wedge of normal epithelium. At last follow-up, her BCVA was 0.8. The normal corneal epithelium extended further by migrating from the periphery toward the center to cover most of the cornea (Fig 1D, E). There was a favorable response to MMC with disappearance of the pigmented conjunctival areas (Fig 1F).

Case 2

A 56-year-old pseudophakic male was referred because of regrowth of a caruncular pigmented lesion in his right eye (Fig 2A).

Fifteen months before his referral, he underwent an incisional biopsy that was diagnosed as benign acquired melanosis without atypia on histopathologic analysis. His slides were reviewed at the hospital and were found to contain areas of atypia for which it was decided to treat him with topical MMC.

Before topical chemotherapy was started, his BCVA was 0.7. He was managed by a total of 3 courses of 0.04% MMC as previously described. On completion of his last MMC cycle, his visual acuity was 0.2, and 2 weeks later, it dropped down to counting fingers at 40 cm. An irregular conjunctivalized epithelium had developed over the entire cornea (Fig 2B). Treatment with topical corticosteroids and nonpreserved tear substitutes was started. During the following 30 months, the condition remained mostly stable with fluctuations of the visual acuity from counting fingers at 40 cm to 0.4. The corneal epithelium presented with areas of irregular epithelium and nonhealing recurrent epithelial defects, requiring a soft bandage contact lens for 2 months. During this time, impression cytologic analysis demonstrated goblet cells in the corneal epithelium, and migrating epithelial cells were seen growing toward the cornea, indicative of LSCD (Fig 2C). Thirty-three months after the last MMC cycle, gradual improvement in his BCVA to 0.7 was noted. Clinically, the areas of irregular hazy epithelium became smaller.

Discussion

Topical chemotherapy with MMC is a well-described treatment for various ocular surface neoplasia. Most of the studies have found it to be a safe and effective treatment, and only rarely to cause permanent damage to the corneal epithelium. This study for the first time reports a relatively high incidence of LSCD (23.8%) occurring after prolonged treatment with topical MMC for PAM with atypia. Older age and longer treatment periods were found to be significant risk factors for this complication. The diagnosis of LSCD was based on the typical clinical appearance of the fluorescein staining pattern and on impression cytologic analysis of the perilimbal epithelium.

Several studies have reported on various complications after topical MMC application. The largest series on complications of MMC therapy for ocular surface neoplasia¹⁸ included 100 patients, most of whom were treated with topical 0.04% MMC 4 times daily using a week-on and week-off regimen for 1 to 3 cycles. Only 3 patients in that

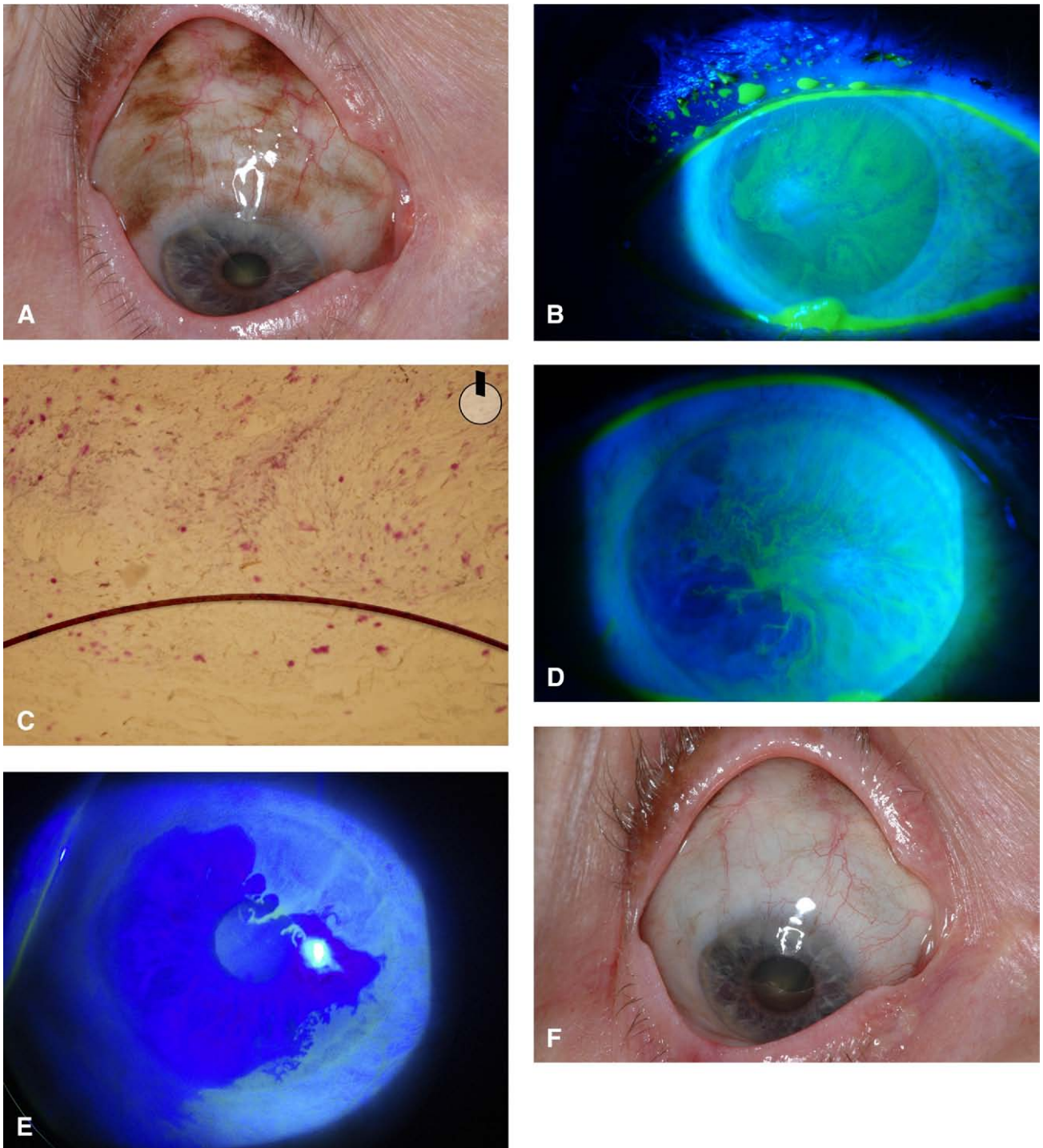


Figure 1. Case 1. **A**, Photograph showing a diffuse pigmented lesion of the right eye involving the bulbar conjunctiva and upper fornix with pigmented cell migration into the cornea before topical mitomycin C (MMC) treatment was started. **B**, Fluorescein staining of the cornea showing the irregular conjunctivalized epithelium with a small wedge of healthy epithelium at the temporal side. **C**, Impression cytologic analysis showing goblet cells invading the corneal epithelium area past the limbus. The black line marks the limbus. The drawing on the right upper side shows the orientation of the filter paper on the cornea and conjunctiva. **D**, Fluorescein staining of the cornea showing gradual extension of a normal corneal epithelium from the temporal side toward the center. **E**, Fluorescein staining of the cornea showing further extension of a normal corneal epithelium covering almost two thirds of the corneal surface. **F**, Clinical photograph obtained at last follow-up showing the excellent response of primary acquired melanosis (PAM) with atypia to topical chemotherapy with MMC.

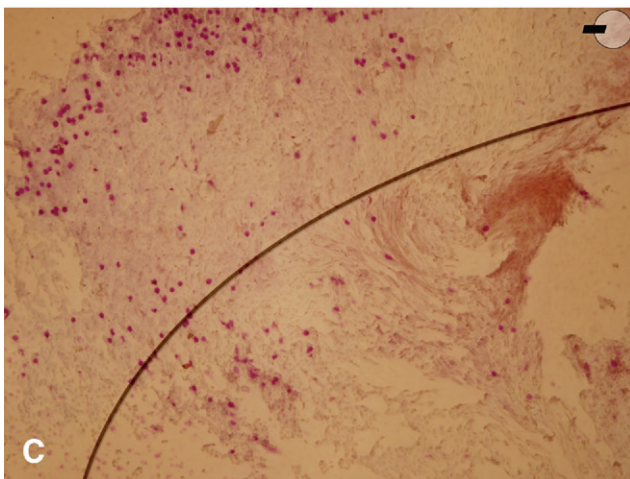
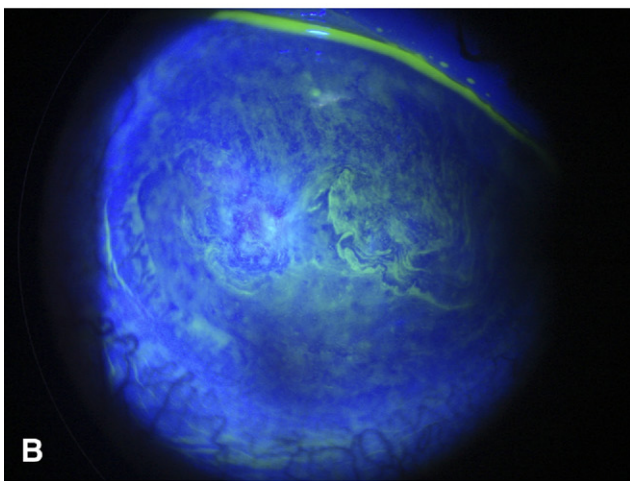
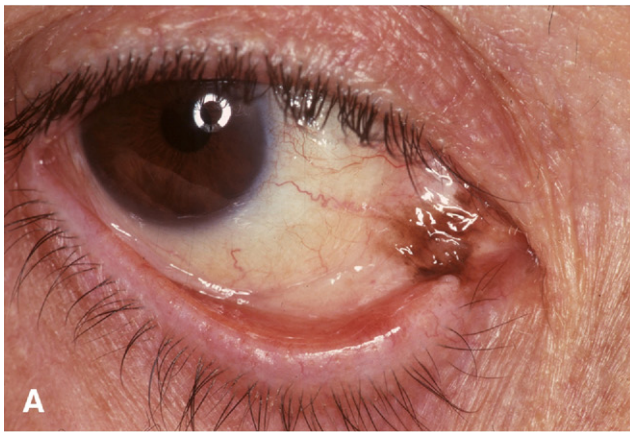


Figure 2. Case 2. **A**, Photograph showing a caruncular pigmented lesion diagnosed as primary acquired melanosis (PAM) with atypia. **B**, Fluorescein staining showing the marked epithelial irregularity over the entire cornea. **C**, Impression cytologic analysis showing goblet cells over the corneal surface, with migrating epithelial cells from the limbal area toward the cornea. The black line marks the limbus. The drawing on the right upper side shows the orientation of the filter paper on the cornea and conjunctiva.

study had PAM with atypia. The common complications reported were allergic reactions (34%) and epiphora because of punctal stenosis (14%). Although the concentration of MMC was the same as what was used for the current patients, the total dose received in that report was much smaller, usually half the dose administered to the present patients. This may suggest that the length of the treatment and cumulative dosage may play a role in the development of LSCD and not only the drug concentration. Our study found the length of treatment to be a marginally significant risk factor for developing LSCD: the mean cumulative treatment period in patients in whom LSCD developed was 78 days, twice that in patients in whom LSCD did not develop (37 days).

Most of the reported complications of topical MMC for ocular surface neoplasia usually are mild, such as conjunctival hyperemia^{8,15–17} and punctal stenosis.^{23,24} Only 2 case reports described serious complications: 1 case of an intumescent cataract²⁵ and 1 case of LSCD.¹⁹

In the only previously reported case of LSCD after topical MMC, the regimen consisted of a total of 5 1-week courses of 0.04% MMC 4 times daily, separated by 1 week-off treatment periods. The patient was a 92-year-old female, and the diagnosis of LSCD was based on the clinical appearance of recurrent corneal erosions and stromal haze¹⁹ and was not confirmed by impression cytologic analysis. The patient's old age and the relatively prolonged treatment time with MMC bear resemblance to the characteristics of the current patients.

Although not specifically diagnosed as LSCD, there are certain clues in the literature that this complication might have occurred after topical MMC, and that its incidence may be greater than previously reported. Complications such as the presence of recurrent corneal abrasions and peripheral corneal pannus,¹⁶ epithelial toxicity,^{9,10} corneal haze,^{7,13} and corneal punctate epithelial keratopathy^{11,17} all could be manifestations of LSCD. It is plausible that at least a few of these cases represent unrecognized LSCD, making this complication underdiagnosed. The authors used impression cytologic analysis to confirm the clinical diagnosis of LSCD and demonstrated the typical signs of invasion of goblet cells into the corneal surface and the migration of epithelial cells through the limbal area into the cornea (Figs 1C and 2C).

An important observation of this study is that LSCD after topical MMC may resolve spontaneously with time, as seen in 3 of the current patients. This outcome was demonstrated in the corneal epithelium fluorescein staining pattern and in the improved visual acuity. This outcome may be a result of a partial damage to the limbal epithelial stem cells, which may recover with time, as the frequent course of repeated MMC cycles is discontinued. Therefore, a conservative approach, including topical corticosteroids for ocular surface inflammation, preservative-free tear replacements, and bandage soft contact lenses for recurrent erosions, may be sufficient in these cases.

Ocular surface neoplasia usually presents a serious treatment challenge and is potentially dangerous. The use of topical MMC has considerably improved the cure of lesions such as PAM with atypia, conjunctival melanoma, and

Table 2. Comparison of Variables between Patients with Limbal Stem Cell Deficiency after Mitomycin C and Patients Who Did Not Have Limbal Stem Cell Deficiency after Mitomycin C (Controls)

	No. of Patients	Age at Diagnosis (yrs)	Male-to-Female Ratio	Best-Corrected Visual Acuity at Diagnosis	Best-Corrected Visual Acuity at Last Visit	Days Receiving Mitomycin C	Follow-up (mos)
LSCD	5	61.8±12.7	1:4 (20%)	0.84	0.54	78.4±24.8	24.8
Controls	16	43.7±16.12	6:10 (37.5%)	0.91	0.78	37.7±3.1	30.62
P value		0.025*	0.62†	0.1*	0.05*	0.07‡	

LSCD = limbal stem cell deficiency.

*Student t test.

†Fisher exact test.

‡Mann-Whitney U test.

CCIN and has provided an effective method of complete tumor removal without the need for surgery. However, the prolonged use of high doses of MMC may be associated with a high incidence of LSCD. Because topical chemotherapy with MMC still is favored as the preferred treatment for ocular surface neoplasia, the authors believe that the protocol of MMC should be modified. This may include reducing MMC concentration to 0.03%, reducing the overall number of treatment courses, or reducing the length of each course. In addition, the authors advocate the use of fluorescein staining and impression cytologic analysis to diagnose LSCD. Increased awareness with this serious adverse effect and modifications of MMC protocol for ocular surface neoplasia will improve the safety of this important treatment method.

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Department of Ophthalmology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel.

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Correspondence:

Abraham Solomon, MD, Department of Ophthalmology, Hadassah-Hebrew University Medical Center, POB 12000, Jerusalem 91120, Israel.

E-mail: aviso@hadassah.org.il.