5. Stein G, Schöne S, Geinitz D, et al. No tissue level abnormality of vitamin A concentration despite elevated serum vitamin A of uremic patients. *Clin Nephrol*. 1986;25:87-93.
6. Yatzidis H, Digenis P, Fountas P. Hypervitaminosis A accompanying advanced chronic renal failure. *Br Med J*. 1975;3:352-3.

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Clinicopathologic case reports of *Alternaria* and *Fusarium* keratitis in Canada

Fungal keratitis is prevalent in tropical environments, making up 35% of all keratitis cases in Florida.[1] However, few cases have been reported in more temperate regions such as Canada.[1–3] In this article, we present 2 Canadian farmers who experienced keratitis involving a dematiaceous fungus, *Alternaria alternata*, and a *Fusarium* species, respectively.

Case 1

In a farming accident, a 49-year-old male sustained a severe alkali injury to his left eye (OS), reducing his visual acuity to 20/120 (Fig. 1A). He developed a hypopyon, which slowly improved on topical 1% prednisolone acetate and oral prednisone (50 mg daily) while continuing with prophylactic topical moxifloxacin and tobramycin. His corneal opacification progressed to an inferior descemetocele. Four months after presentation, he received a Boston keratoprosthesis (k-pro).

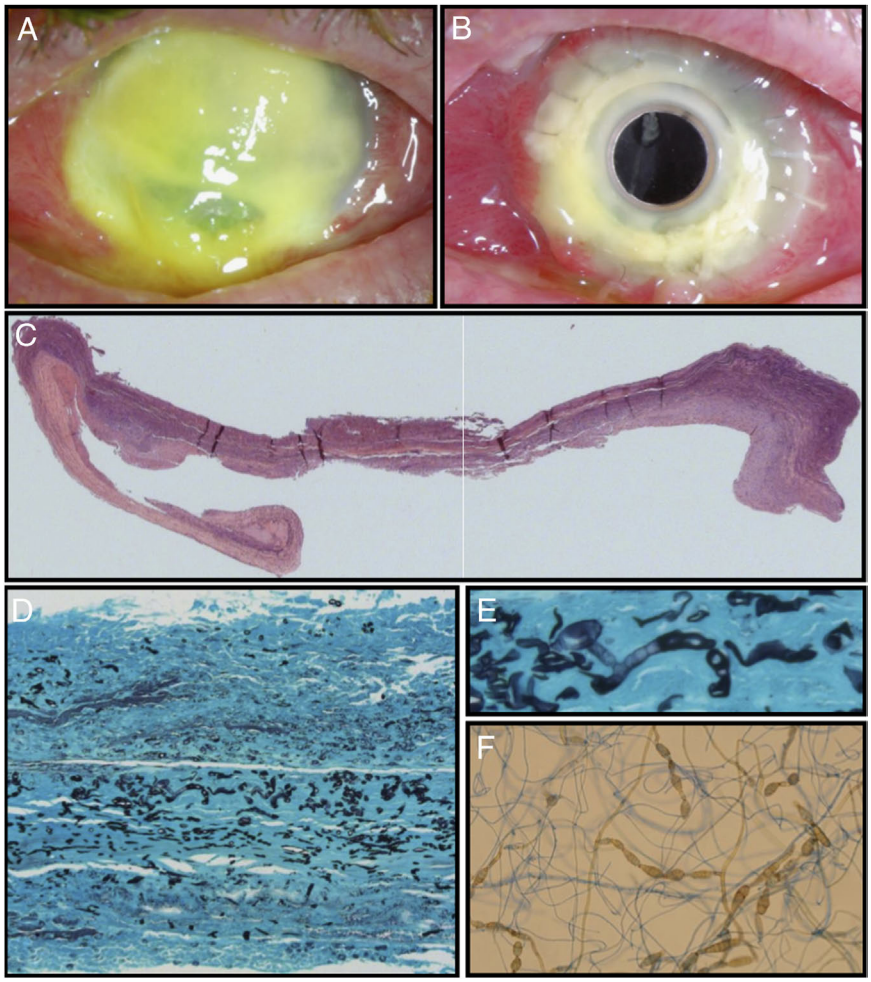

Fig. 1—A, Corneal ulceration after alkaline chemical burns to the left eye. B, One month after k-pro implantation with the infectious process infiltrating into the graft material. C, The corneal specimen is severely necrotic with irregular thinning of the inflamed stroma (hematoxylin and eosin, ×25 original magnification). D, The Grocott–Gomori methenamine silver stain (GMS) shows numerous filamentous fungi at all levels predominantly anteriorly in the necrotic central stroma (×200 original magnification). E, Higher magnification of septated fungi (GMS, ×640 original magnification). F, Tease mount of a 72-hour culture growth that has been stained with lactophenol cotton blue, showing large, brown pigmented, muriform macroconidia occurring in short chains that is characteristic of *Alternaria* species (×640 original magnification).

Routine cultures were negative. One month later, he returned with a wound leak and an infiltrate into the graft, which cultured positive for *A. alternata* (Fig. 1B). He was treated with intracameral amphotericin B, along with topical amphotericin B, moxifloxacin, and vancomycin, and oral itraconazole and ciprofloxacin. Collagen cross-linking (CXL) with 0.1% riboflavin was administered 3 days later. The infection continued, causing melting around the k-pro beyond the limbus. Two months after transplant, he underwent sclerokeratoplasty. Cultures of the k-pro isolated *A. alternata.* Despite continued topical and oral antimicrobials, the eye experienced development of culture-negative endophthalmitis 1 month postoperatively and became phthisical.

Examination of the initial corneal specimen showed extensive necrosis and a severe nongranulomatous inflammatory infiltrate (Fig. 1C). The epithelium, Bowman layer, endothelium, and most of Descemet membrane were absent. Numerous septated filamentous fungi (Fig. 1D, 1E) extended to 1 specimen margin. The tease mount showed large, brown, muriform macroconidia occurring in short chains, characteristic of *Alternaria* species (Fig. 1F).

Case 2

A 40-year-old male soft contact lens (CL) wearer presented with hand-motion vision and a 10-day history of a left central corneal ulcer (Fig. 2A). The patient was a farmer who had been combating a *Fusarium* infection in his wheat crop. Corneal cultures yielded *Fusarium* species, which was treated with 0.15% drops amphotericin B hourly, moxifloxacin drops every 2 hours, and oral ketoconazole 200 mg twice daily. The corneal ulcer persisted and a repeat culture 4 weeks after presentation isolated *Fusarium* species. The cornea was collagen cross-linked with 0.1% riboflavin 1 week later. The patient returned 6 days later with pain and a hypopyon, at which time voriconazole drops were instilled followed by a short course of topical 1% prednisolone acetate. The hypopyon improved, but the ulceration failed to stabilize and the cornea perforated centrally 4 weeks later, which was managed by a sclerokeratoplasty with perioperative intracameral voriconazole. At 3 months of follow-up, the patient's visual acuity improved to 20/60. He subsequently experienced graft rejection with no evidence of infection reactivation.

Histopathologic examination of the necrotic cornea showed marked central thinning with perforation (Fig. 2B, 2C). There were extensive acute and chronic nongranulomatous inflammatory infiltrates throughout, especially paracentrally. The epithelium and Bowman layer were absent. Descemet membrane was fragmented centrally and the endothelium was severely attenuated.

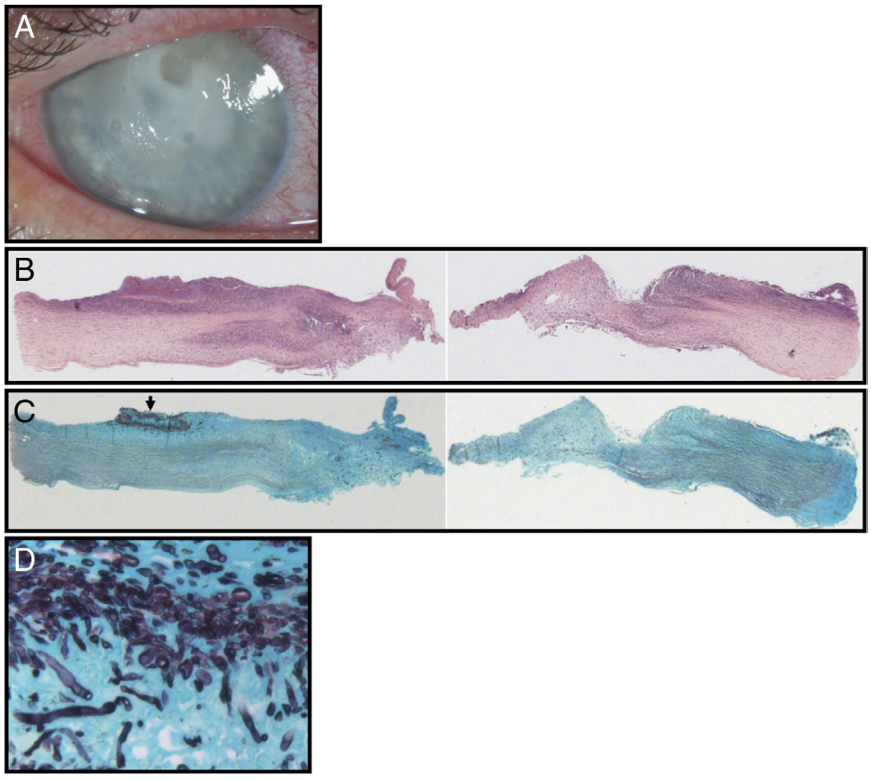

Fig. 2—A, Corneal ulceration from *Fusarium* infection of more than 10 days' duration in the left eye. B, The cornea is markedly thinned and inflamed centrally with superficial ulceration and a central perforation (hematoxylin and eosin, ×25 original magnification). C, The Grocott–Gomori methenamine silver stain (GMS) shows fungi mainly paracentrally with a large patch superficially (*arrow*) toward one side (×25 original magnification). D, Large aggregates of filamentous septated fungi in the superficial stromal in the region of the *arrow* in Figure 1C (GMS, ×640 original magnification).

Septated filamentous fungi were found full thickness centrally and in the anterior half of the cornea peripherally, consistent with *Fusarium* species (Fig. 2C, 2D).

Discussion

Alternaria and *Fusarium* species are opportunistic pathogens commonly found in the soil and in plants,[3,4] with individuals in the agricultural industry being especially at risk for acquiring these infections after trauma. Geographic location greatly influences the prevalence of fungal keratitis, decreasing to 2% in more temperate areas such as New York.[1] *Candida* species are the major causative agent in the northern United States, whereas *Fusarium* species are more frequent in warmer regions.[1] To our knowledge, Case 1 is the first report of *A. alternata* keratitis in Canada.

A number of factors increased the risk for fungal infection in Case 1. This patient was a poor candidate for lamellar and penetrating keratoplasty because of the extensive chemical damage with resultant cicatrizing conjunctivitis and limbal stem cell deficiency. K-pro is well tolerated in these unfavourable post–chemical injury corneas.[5,6] However, the combination of a poor ocular surface and the synthetic k-pro optic likely encouraged formation of a fungal biofilm and invasion into and around the graft leading to inflammation-mediated stromal melting. Thus, in these high-risk cases, prophylactic antifungal therapy also may be considered. Chan and Holland[7] found that in patients with k-pro, CL and systemic immunosuppression were not predisposing factors to infection. Moreover, collagenase and proteinases are released after an alkaline injury, promoting corneal melting. Melting may have been further compounded by the use of topical corticosteroids, which may promote collagenases activity.[8]

Fungal infections are best controlled with early treatment. Case 2 presented 10 days after the onset of infection. This late presentation is associated with an increased requirement for penetrating keratoplasty (PKP).[9] Furthermore, in *Fusarium* keratitis involving the deeper stroma, meta-analysis found that 70% were unsuccessful on medical therapy alone.[4] CXL has been reported to yield good outcomes in infectious keratitis, controlling and preventing emergency PKP.[10] CXL increases corneal integrity, making the collagen matrix more resistant to digestive enzymes released by infectious pathogens, as well as promoting a direct antimicrobial effect.[10,11] However, CXL also may reduce drug penetration.[12] In a porcine model of CXL, the reduction in penetration for voriconazole was 15.6%.[12] It is important to consider that CXL in Case 2 may have been unsuccessful because the tissue was extensively infiltrated with fungi. CXL-mediated fungal death may have triggered an inflammatory response, and in combination with inadequate drug penetration after CXL treatment, led to complications resulting in an emergent PKP for corneal perforation.

Although in Case 2 the PKP failed because of rejection, PKP is effective for controlling fungal keratitis.[13] Case 2 was maintained on antifungal medication following the control of the infection after the PKP. It is unclear whether the use of voriconazole along with the PKP significantly contributed to the initial successful outcome, but it also has been suggested that the PKP promotes the absorption of topical voriconazole.[14]

Despite its relative rarity in the cooler Canadian environment, ophthalmologists should maintain a high index of suspicion toward fungal keratitis, especially in traumatic or CL-related keratitis that does not improve on broad-spectrum antibiotics. Furthermore, both *Fusarium* and *Alternaria* species are common North American crop pathogens.[15] It is important that both farmers and physicians are aware of the potential hazard of fungal keratitis to minimize its occurrence and initiate appropriate therapy in a timely manner.

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References

1. Hassan HM, Papanikolaou T, Mariatos G, et al. Candida albicans keratitis in an immunocompromised patient. *Clin Ophthalmol*. 2010;4:1211-5.
2. Jurkunas U, Behlau I, Colby K. Fungal keratitis: changing pathogens and risk factors. *Cornea*. 2009;28:638-43.
3. Zahra LV, Mallia D, Hardie JG, et al. Case report. Keratomycosis due to Alternaria alternata in a diabetic patient. *Mycoses*. 2002;45:512-4.
4. Thomas PA. Current perspectives on ophthalmic mycoses. *Clin Microbiol Rev*. 2003;16:730-97.
5. Zerbe BL, Belin MW, Ciolino JB. Results from the Multicenter Boston Type 1 Keratoprosthesis Study. *Ophthalmology*. 2006;113:1779.e1-7.
6. Basu S, Taneja M, Narayanan R, et al. Short-term outcome of Boston Type 1 keratoprosthesis for bilateral limbal stem cell deficiency. *Indian J Ophthalmol*. 2012;60:151-3.
7. Chan CC, Holland EJ. Infectious keratitis after Boston type 1 keratoprosthesis implantation. *Cornea*. 2012;31:1128-34.
8. Davis AR, Ali QK, Aclimandos WA, et al. Topical steroid use in the treatment of ocular alkali burns. *Br J Ophthalmol*. 1997;81:732-4.
9. Rautaraya B, Sharma S, Kar S, et al. Diagnosis and treatment outcome of mycotic keratitis at a tertiary eye care center in eastern India. *BMC Ophthalmol*. 2011;11:39.
10. Anwar HM, El-Danasoury AM, Hashem AN. Corneal collagen crosslinking in the treatment of infectious keratitis. *Clin Ophthalmol*. 2011;5:1277-80.
11. Spoerl E, Wollensak G, Seiler T. Increased resistance of crosslinked cornea against enzymatic digestion. *Curr Eye Res*. 2004;29:35-40.
12. Tschopp M, Stary J, Frueh BE, et al. Impact of corneal cross-linking on drug penetration in an ex vivo porcine eye model. *Cornea*. 2012;31:222-6.
13. Xie L, Dong X, Shi W. Treatment of fungal keratitis by penetrating keratoplasty. *Br J Ophthalmol*. 2001;85:1070-4.
14. Jurkunas UV, Langston DP, Colby K. Use of voriconazole in the treatment of fungal keratitis. *Int Ophthalmol Clin*. 2007;47:47-59.

15. Lacey J, Ramakrishna N, Hamer A, et al. Grain fungi. In: Arora DK, Mukerji KG, Marth EH, eds. *Handbook of Applied Mycology. Volume 3: Foods and Feeds.* New York: Marcel Dekker, 1991:121-78.

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Pigmented conjunctival growing lesion in a teenager: nevus or melanoma?

Conjunctival melanoma (CM) is a rare tumour (0.06–0.74 case/million[1,2]), with a 10-year melanoma-related mortality rate around 30%.[1,3] It arises from epithelial melanocytes and may develop from primary acquired melanosis, pre-existing nevus, or de novo in white adults. The spectrum of these tumours differs in the pediatric age group, which are predominantly benign nevi rarely evolving into melanoma.[4] We present a case of a teenager with a growing pigmented conjunctival lesion, with pathologic characteristics of malignancy.

A 15-year-old male presented with a conjunctival lesion since childhood that showed growth during the previous year (Fig. 1A–C). Ocular examination revealed an 11 × 9-mm temporal pigmented mass, adjacent to the limbus. Excisional biopsy was proposed, but parents rejected that option. Two months later, the mass showed nodular growth in addition to basal growth (Fig. 1D). Wide microsurgical excisional biopsy with 3-mm free margins was performed, working with the "no-touch" technique.

Microscopic examination showed a nodular lesion composed of a proliferation of confluent atypical epithelioid cells nests with nuclear pleomorphism involving full-thickness conjunctival epithelium with ulceration (Fig. 2A, 2B). It also extended into the underlying stroma, with lack of maturation, focal dense lymphocytic infiltrate, and epithelial inclusion cysts. Surgical margins were free. Immunohistochemically, the tumour cells stained for melanocytic markers HMB-45 (Fig. 2C), S100, and Melan A. The Ki-67 growth fraction ranged from 10% to 25% (Fig. 2D). This marker has an accepted role in distinguishing benign from malignant lesions. According to Jakobiec et al.,[5] melanomas display more than 10% nuclear positivity among all cells counted, whereas current nevi display approximately 1%.

First diagnosis was juvenile conjunctival nevus (JCN) with atypical cells versus CM. Based on the development of ulceration, HMB-45 positivity, and the high proliferation index, the lesion as described represents a malignant transformation of a conjunctival nevus. The term *melanoma in situ* is not used anymore.

Oncologic examination was performed obtaining negative results for regional lymph nodes and systemic extension by CT body scan including neck, chest, and abdomen examination. Ten months after biopsy, no signs of recurrence or systemic extension were found.

CM is rare in children. In Triay et al.'s[1] series of CM (170 cases), all patients were older than 35 years at the diagnosis time, except 2 of them, aged 20 and 22 years. In Shields and Shields'[4] review (1643 cases), no patients with CM were younger than 20 years. Taban and Traboulsi,[6]

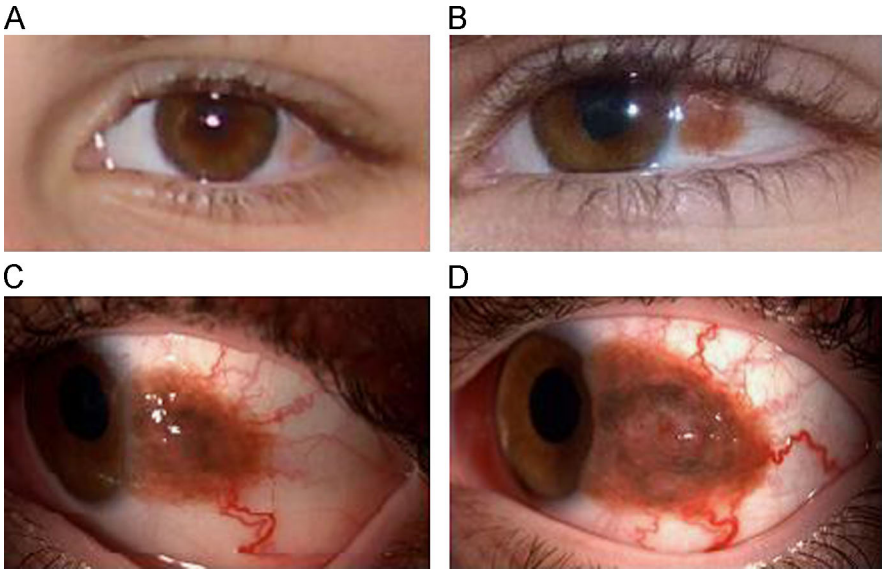

Fig. 1—A, Pigmented temporal flat conjunctival lesion 7 years before examination. B, Two years later, lesion increased slightly in size and pigmentation. C, Conjunctival pigmented and elevated mass, reaching the limbus, with marked vascularization at first ocular examination. D, Two months later, it presented evident growth with conjunctival ulceration.