 Conjunctival melanoma is a rare condition of the eye pigment predominantly affecting white adults. We describe a 32-year-old white man with an amelanotic malignant melanoma of the conjunctiva that is not associated with primary acquired melanosis (PAM) or melanocytic nevus.

The patient presented with a 2-year history of nonpigmented vascularized nodules of the right eye. Results of hematoxylin and eosin (H&E) staining of the lesion showed an invasive nodule with vertical spreading, invasion of the substantia propria corneae, and ulceration. S100 protein was expressed in the cells of the invasive nodule. HMB45 protein was highly positive in the melanoma cells. The de novo amelanotic malignant melanoma of the conjunctiva we describe is an extremely uncommon tumor mainly affecting white adults.

Cutis. 2006;77:377-381.

Conjunctival melanoma accounts for 2% to 3% of all ocular tumors in white adults.\textsuperscript{1,2} Invasive malignant melanoma develops in approximately 50% of patients with primary acquired melanosis (PAM) with atypia. Clinically, PAM is a unilateral condition that is characterized by diffuse speckled pigmentation of varying intensity occurring anywhere on the conjunctiva. It also may involve the caruncle and corneal epithelium. Histologically, the presence of intraepithelial melanin deposition alone or with melanocytic hyperplasia without cytologic atypia is designated as PAM without atypia. These lesions rarely progress to malignant melanoma. By contrast, the clinical appearance of a unilateral flat brown patch in the conjunctiva may be the result of an intraepithelial melanocyte with melanocytic atypia, a condition known as PAM with atypia, which eventually progresses to melanoma.

A smaller number of conjunctival melanomas evolve from a preexisting nevus and appear de novo. Conjunctival melanomas that occur de novo are probably the least common, whereas histopathologic examination results show no signs of PAM or nevi in 12% of conjunctival melanomas.\textsuperscript{3,4} In some cases, it is possible that the precursor lesions may not be recognized and, in fact, de novo presentation may be rare.\textsuperscript{5} The crude and age-standardized annual incidence rate is 0.51 to 0.54 per million people. The age-specific incidence is 0.06 for patients younger than 30 years, 0.48 for patients aged 30 to 49 years, 1.05 for patients aged 50 to 70 years, and 1.57 for patients older than 70 years.\textsuperscript{5} Epidemiologic data indicate that melanoma of the skin is 450 to 900 times more common than conjunctival melanoma.\textsuperscript{6} We report a case of an unsuspected amelanotic conjunctival melanoma that occurred de novo and was diagnosed on histologic evaluation.

Case Report
In March 2003, a 32-year-old white man presented to our dermatology department with nonpigmented vascularized nodules of the right eye of 2 years’ duration (diameter, 10 mm; elevation, 3 mm) (Figure 1). The malignant melanoma involved the limbal area and bulbar conjunctiva (Figure 2). The lesions arose from an area without clinically detectable PAM or pigmentation. Results of an examination of the ipsilateral submandibular, preauricular, and cervical lymph nodes were negative. The lesion was excised with local resection by a dermatologic surgeon. The patient is in follow-up to monitor local recurrence and evidence of systemic spread.

The biopsy specimen was fixed in 10% buffered formalin and embedded in paraffin; hematoxylin and eosin (H&E)–stained sections from the specimen were examined. Immunophenotypic studies that included positive and negative controls were performed. Sections measuring 4 µ were taken from the 10% buffered formalin and paraffin-embedded
Amelanotic Conjunctival Melanoma

tissue blocks and were placed on silane-coated glass slides, deparaffinized, and then rehydrated. The slides were pretreated in a citrate buffer solution (pH 6.1) heated at 120°C for antigen retrieval. The sections subsequently were treated with 3% hydrogen peroxide to block endogenous peroxidase activity and then with 10% normal horseradish peroxidase incubated for 20 minutes at 20°C in a humid chamber. The primary antibodies used in this study were monoclonal to S100 and HMB45 proteins. The primary antibodies were incubated for 30 minutes at room temperature. Immunostaining was performed by avidin-biotin complex technique. Antigen-antibody reaction was visualized by 3,3’-diaminobenzidine.

The results of H&E staining of the lesion showed an invasive nodule with vertical spreading, rapid invasion of the substantia propria corneae, and ulceration (Figure 3). The tumor cells showed great variation in size and shape with atypia and nuclear pleomorphism (Figure 4). Two major types of cells could be recognized: epithelioid and spindle. The neoplastic cells grew in a sheetlike patternless fashion, with segregate nests and cells. The upward extension of tumor cells in the conjunctiva led to disintegration and ulceration of the smooth, flexible, protective sac of the conjunctiva. The melanoma did not have an intraepithelial component extending far beyond the margins of the deepest part. There was no relationship with nevus

Figure 1. Arrows point to nonpigmented vascularized nodules of the right eye.

Figure 2. Malignant melanoma involving the limbal area and bulbar conjunctiva. Arrows point to limbal area and bulbar conjunctiva.
melanocyte cells. An inflammatory infiltrate was intermingled with neoplastic cells. The number of mitotic figures was small. There was no evidence of melanin in the H&E stain results.

Immunohistochemical staining for S100 and HMB45 proteins was a useful diagnostic tool for defining the extension of the lesion and the presence or absence of PAM. S100 was expressed in the cells of the conjunctival melanoma. In some areas of the lesion, there was a reduction of S100 levels. HMB45 was highly positive in the melanoma cells (Figure 5). There was variation in the intensity of staining for HMB45 (Figure 6). In the same sections, the tumor had both positive and negative cells, and some nodules were more positive for HMB45 than others.

**Comment**

The conjunctiva is a mucous membrane lined by an epithelium that contains goblet cells; the stroma lacks dermal stratification. The melanocytes in the conjunctiva and uveal tract are similar to the melanocytes in the skin. They typically are solitary and dendritic with fine ovoid melanin granules; sizes and frequency vary by race. These melanocytes are considered to be the precursor cells of melanoma.
The diagnostic challenge in melanocytic lesions of the conjunctiva is both clinical and histologic. The clinical objective is to identify patients at risk for progression to melanoma, particularly those with PAM. Clinical clues include color, movability over the globe, and the edges and thickness of the lesion; however, these clues are not reliable. Usually, the features of an invasive melanoma are those of a pigmented, smooth, vascularized limbal nodule; in rare cases, the growth may be pedunculated.

The histology of skin and conjunctival melanomas is noncomparable in all features. In particular, the conjunctiva does not contain a papillary dermis. There is not a definitive method of quantifying vertical growth in the conjunctiva. Therefore, any melanocytic lesions that invade the substantia propria corneae must be suspected as malignant melanoma.

Conjunctival melanoma is a rare condition of the eye pigment predominantly affecting white adults. Any pigmented lesion of the conjunctiva poses difficulties in clinical and histologic diagnoses and should be considered a probable melanoma. A further difficulty is the occurrence of amelanotic

**Figure 6.** Immunohistochemical staining shows variation in the intensity of staining for HMB45 in the melanoma cells (original magnification ×640).
Amelanotic Conjunctival Melanoma

lesions in which the melanocytic nature of the tumor may not be understood from the results of clinical observations and routine stains.

This report describes a 32-year-old white man with amelanotic malignant melanoma of the conjunctiva not associated with PAM or melanocytic nevus. Absence of conjunctival pigmentation in the melanoma prevented early clinical detection of this lesion. The lack of pigmentation also makes clinical diagnosis virtually impossible, and diagnosis can be established only immunohistopathologically. 10

Immunohistochemical staining was a useful diagnostic tool and helped to establish the extent of the lesion and to exclude the presence of PAM and melanocytic nevus. S100 and HMB45 positivity in the neoplastic lesion was valuable in confirming the diagnosis of the amelanotic conjunctival melanoma de novo. 11,12

A conjunctival melanoma may be simulated by numerous lesions, such as conjunctival nevi, racial melanosis, conjunctival ephelides, and PAM both with and without atypia. For example, a melanotic lesion confined to the epithelium in an adult patient with recent onset is more likely to be PAM; in a younger patient, junctional nevi is more probable. 13,14 Results of a biopsy yield more information diagnostically and prognostically.

The de novo amelanotic malignant melanoma of the conjunctiva we describe is an extremely uncommon tumor that affects white adults. Despite recent advances, this neoplasm remains one of the most unpredictable and enigmatic entities in malignant melanomas.

REFERENCES