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Conjunctival Melanocytic Lesions

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- Context.—Conjunctival melanocytic lesions consist of a variety of neoplastic and nonneoplastic conditions. These include benign processes such as primary intraepithelial hypermelanosis and melanocytic hyperplasia, secondary forms of intraepithelial hypermelanosis and melanocytic hyperplasia, melanocytic nevi, melanocytic proliferations with malignant potential, and melanoma.

Objective.—To provide a concise yet comprehensive resource regarding the histopathologic diagnosis of conjunctival melanocytic lesions. We aim to detail and clarify the numerous classification schemes that exist for junctional melanocytic proliferations of the conjunctiva (known as primary acquired melanosis or PAM; also termed conjunctival melanocytic intraepithelial neoplasia or C-MIN). Although not uniformly adopted, C-MIN is classified by using a numeric system based on a defined set of criteria. A less complex scheme (conjunctival melanocytic intraepithelial lesion or CMIL) has recently been proposed by the World Health Organization. Additionally, we aim to update the reader regarding molecular features and prognostic indicators.

Data Sources.—Peer-reviewed literature and archived cases for illustration.

Conclusions.—Accurate histologic classification is essential, as PAM/C-MIN/CMILs that have a significant potential to progress to invasive melanoma may be clinically indistinguishable from low-risk lesions. Conjunctival melanoma (CM) more closely resembles cutaneous melanoma in terms of its pathogenesis and molecular features, compared to melanoma arising at other mucosal sites or to uveal melanoma. Depth of invasion and ulceration status, among other factors, have emerged as important prognostic indicators in CM. Sentinel lymph node biopsy may provide further prognostic information. Lastly, integration of pathologic and clinical findings is essential at this anatomically sensitive location to determine appropriate clinical management.

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The histology of the conjunctiva ranges from a stratified nonkeratinized squamous mucosa to a more columnar epithelium with numerous goblet cells, depending on anatomic location. The subepithelial space (analogous to the dermis in the skin) is termed the substantia propria and is composed of a loose collagenous stroma containing lymphovascular channels. Anatomically, the conjunctiva is divided into 4 zones: the palpebral conjunctiva, which lines the inner surface of the eyelids and contains pseudoglands of Henle, which represent infoldings of the conjunctiva that often resemble glandular structures in histologic sections; the fornical conjunctiva, which lines the superior and inferior fornices; the bulbar conjunctiva, which covers the anterior surface of the globe; and the caruncular conjunctiva medially, in which the underlying stroma contains sebaceous glands, accessory lacrimal glands, and hair follicles. The plica semilunaris, a crescentic fold of conjunctiva, is just lateral to the caruncle and contains abundant goblet cells. Like the skin, there are normal resident melanocytes along the basal layer of the conjunctival epithelium. Melanocytes are greatly outnumbered by epithelial cells; approximately 5 to 15 basal epithelial cells are present for every 1 melanocyte. Although the density of goblet cells varies depending on location, the variability of melanocytic density across different areas of the conjunctiva has not yet been formally assessed. In contrast to skin, no rete ridges are present, and the concepts of papillary and reticular dermis do not apply.

 Conjunctival neoplasms consist of both melanocytic and nonmelanocytic tumors, the majority of which are benign. Most conjunctival neoplasms are melanocytic in nature; however, this calculation may be influenced by referral bias. Conjunctival melanocytic lesions can be separated into 3 broad categories: benign, which includes nevus and primary acquired melanosis (PAM) without atypia/ conjunctival melanocytic intraepithelial neoplasia 0-1 (C-MIN 0-1); low-grade conjunctival melanocytic intraepithelial lesions (low-grade CMILs); lesions with significant risk of progression to melanoma, which include PAM with atypia/ C-MIN 2-5/high-grade CMILs; melanoma in situ (C-MIN >5); and melanoma. Complexion-associated melanosis, ephelides (freckles), and secondary melanosis, which is
seen in some systemic conditions such as Addison disease or as a drug side effect, do not have malignant potential. Although rare, the prevalence of conjunctival melanoma (CM) has been increasing in the United States, Finland, and Sweden. When evaluating pigmented conjunctival lesions, it is important to consider the clinical characteristics, including age at time of presentation, appearance, location (ie, bulbar versus tarsal), and histologic features to determine the most appropriate treatment.

Conjunctival melanocytic lesions can present particular difficulty in precise classification. In addition to being infrequent specimens encountered by the practicing general surgical pathologist or dermatopathologist, conjunctival biopsy specimens are typically very small. Furthermore, specimens may often be tangentially oriented upon embedding or show extensive crush artifact, further limiting histologic evaluation.

The purpose of this review is to summarize the clinical, histologic, and molecular findings in each category of conjunctival melanocytic lesions and to clarify recent updates in terminology, particularly with regard to primary lentiginous junctional melanocytic proliferations of the conjunctiva. These lesions can be classified in a number of ways, generating considerable potential for confusion. Historically referred to as primary acquired melanosis, a more recent but not widely adapted terminology of conjunctival melanocytic neoplasia has been proposed. Other more simplified classification schemes to limit interobserver variability have been suggested, which will also be discussed. Numerous clinical images are included to help orient the pathologist and develop understanding of clinical findings, with the goal of enabling appropriate communication between the pathologist and clinician.

BENIGN EPITHELIAL HYPERMELANOSES OF THE CONJUNCTIVA

Several lesions can be classified as benign epithelial hypermelanoses, which are characterized by an increase in melanin pigment, but without an increase in the number of melanocytes, or only a mild hyperplasia and an absence of melanocytic atypia. Primary conjunctival hypermelanoses include ephelides (freckles), which present as hyperpigmented macules without melanocytic hyperplasia, as well as complexion-related melanosis (formerly termed naevus or nevus of Ota).2,12,13 Ephelides are present in up to 10% of the population and are more common in individuals with Fitzpatrick skin types IV-VI.2,12,13 These lesions are typically bilateral (Figure 1, A). Benign melanocytic involvement of the conjunctiva can also be pigmented.11 Histologically, increased melanin deposition within the epithelium and within the substantia propria, often with clear cysts, intrinsic vasculature or prominent feeder vessels, and corneal involvement or location in the palpebral, caruncular, or fornical conjunctiva.2,12,13 Feeder vessels are present in a minority of CN.2,12

Clinical Features

Conjunctival nevi (CN) are the most common conjunctival neoplasms as well as the most common melanocytic lesions of the conjunctiva.6 Conjunctival nevi, like cutaneous nevi, can be classified as congenital or acquired based on time of appearance. Most CN are thought to represent acquired lesions as they appear later in childhood, during puberty, or early in the third decade of life.2 Conjunctival nevi are more common in the Caucasian population with a mean age at first presentation in the early 30s and with equal sex distribution.8,12,13

Anatomically, CN are most commonly found on the bulbar conjunctiva with similar involvement of the temporal and nasal quadrants of the eye. Less frequently they may be seen in the superior and inferior quadrants of the eye, as well as in the caruncle and plica semilunaris. Conjunctival nevi may be focal or diffuse but are characteristically unilateral and unifocal (Figure 1, C and D).2 Clinically, the pigmentation level of CN is varied and can range from darkly pigmented to amelanotic.8,12 Changes in pigmentation within a given nevus are rare, seen in only 5% of cases, and should prompt clinical concern.8 Likewise, changes in size are only typically seen in 7% of CN.8 Transformation to melanoma is rare.8

The diagnosis of CN is usually made during slit lamp examination, by observing classic clinical features, and occasionally by histopathologic examination. The lesions are well-defined and very often contain microcysts (Figure 1, C), visible with the slit lamp beam. Excisional biopsies should be performed to rule out melanoma after documented tumor growth, recurrence after a previous excision, or for cosmetic concerns.2,12 Clinical features predictive of surgical excision are greater patient age, large basal tumor diameter, presence of clear cysts, intrinsic vasculature or prominent feeder vessels, and corneal involvement or location in the palpebral, caruncular, or fornical conjunctiva.2,12,13 Feeder vessels are present in a minority of CN.2,12

Histologic Features

Depending on the localization of the melanocytes, 3 histopathologic subtypes are described: junctional, compound (Figure 2, A), and subepithelial (the latter being the equivalent of dermal nevi in the skin; Figure 2, B). Junctional nevi, which are generally seen only in children, are characterized by discrete nests of benign nevus cells within the epithelium only. The cells in these junctional nevi may show an epithelioid appearance with some mitotic activity; however, they may not produce enough melanin pigment for the lesions to be easily detected.2,13

A compound nevus contains melanocytes both in the epithelium and within the substantia propria, often with cystic invaginations of surface squamous epithelium containing goblet cells (Figure 2, C and D). Such cysts are seen in most cases; however, they are uncommon in melanoma.14 As such, they can be particularly useful as a feature of benignity if present.2,12,13 As a result of this, compound nevi are often clinically thicker and commonly contain cystic areas.15 Subepithelial nevi only contain melanocytes within the substantia propria and thus are analogous to intradermal nevi in the skin. In addition to cysts, CN can show a
range of cytologic features and can contain balloon cells and spindle cells. Just as nevomelanocytes mature and become smaller with descent into the substantia propria as well as lose their ability to produce pigment (except in dark-skinned individuals), subepithelial nevi are the most likely to be amelanotic. Although maturation is present in many nonjunctional nevi and its presence is a reassuring feature, reverse maturation, in which the subepithelial cells are larger than those in the junction, is commonly seen in CN occurring in children and should not necessarily be interpreted as evidence for malignancy. Additional features often present in children include a confluent junctional component and a prominent inflammatory infiltrate. Additionally, with respect to maturation of the subepithelial component, many CN may only demonstrate subtle features of this phenomenon in comparison to cutaneous nevi, as seen in Figure 2, A and B. Figure 2, D, which represents a caruncular nevus, shows well-developed maturation more akin to that seen in cutaneous nevi.

One histologic variant of conjunctival nevus that can cause diagnostic confusion has been referred to as inflamed juvenile conjunctival nevus. These lesions are typically found in the adolescent age group and are associated with a clinical history of allergic disorders. They are usually found near the limbus and demonstrate periodic congestion and growth, which may cause clinical concern. Common histologic features may also give rise to overinterpretation as an atypical or malignant lesion, such as a dense inflammatory infiltrate that may contain eosinophils but also with enlarged individual subepithelial histiocytes that can be mistaken for melanocytes.

Other variants of conjunctival nevus include heavily pigmented lesions such as blue nevus of the conjunctiva, cellular blue nevus, and deep penetrating nevus (DPN). Blue nevi, which present clinically in the conjunctiva as dark brown to black lesions due to the absence of the Tyndall effect, are usually slightly raised in appearance. Together with their clinical appearance and their mean age of presentation in the sixth decade of life, these lesions can be clinically worrisome for PAM/C-MIN/CMIL or melanoma. Analogous to their cutaneous counterparts, conjunctival blue nevi typically contain a subepithelial proliferation of dendritic to spindled melanocytes with bland cytologic features. As in the skin, combined nevi with a blue nevus component are occasionally encountered (Figure 2, F). Extremely rare examples of cellular blue nevi have also been described. Deep penetrating nevi of the conjunctiva are occasionally encountered and usually occur in combination with a common nevus component. Similar to their cutaneous counterparts, conjunctival DPNs are typically predominantly subepithelial and are composed of plump growth.
Figure 2. Histologic variation of conjunctival nevi. A, Compound nevi are characterized by nevomelanocytes present in both the epithelium and substantia propria. B, Subepithelial nevi are characterized by nevomelanocytes only present in the substantia propria and not in the epithelium. These lesions are thought to be analogous to intradermal nevi in the skin. C and D, Cystic invaginations of the surface epithelium are often associated with conjunctival nevi. Their presence, however, is not always indicative of benignity. Note the convincing evidence of histologic maturation in (D) with descent into the substantia propria, with deeper type C melanocytes showing a neural appearance in this caruncular nevus. E, Balloon cell change is a common phenomenon in conjunctival nevi. F, This combined subepithelial nevus contains a blue nevus component composed of spindled pigmented melanocytes that is seen in the deeper substantia propria (hematoxylin-eosin, original magnifications ×200 [A through D and F] and ×400 [E]).
spindled to epithelioid melanocytes with finely pigmented gray cytoplasm disposed in nests and cords. Prominent background melanophages are present, resulting in a darkly pigmented clinical appearance. Such lesions can cause considerable diagnostic confusion, as some degree of cytologic atypia and lack of complete maturation with descent into the substantia propria are commonplace. Additionally, in combined lesions, the cells of the DPN component are usually larger than those of the common nevus component, leading to concern for melanoma arising in a preexisting nevus.21 Also included in this category of pigmented conjunctival melanocytic lesions is conjunctival pigmented epithelioid melanocytoma, a rare heavily pigmented melanocytic neoplasm of indeterminate biologic potential containing a cellular proliferation of epithelioid to spindled melanocytes analogous to those seen in the skin.22

Granular cell change, which may be caused by accumulation of degraded melanosomes, is a cytologic phenomenon that has rarely been observed in CN. As nevomelanocytes with granular cell change are epithelioid in appearance, the presence of this phenomenon can lead to overinterpretation as melanoma.10,23 As studies reporting the presence of this phenomenon did not evaluate nevi with granular cell change with immunohistochemistry for β-catenin, it is possible that this cytomorphology exists along the spectrum of DPN, considering the lack of maturation and observed expression of HMB-45.

Conjunctival Spitz nevi are exceedingly rare, occur primarily in children, and are histologically analogous to their cutaneous counterparts.2 Clinically, Spitz nevi can undergo rapid growth and present as nonpigmented raised lesions. Histologically, lesions are typically well-circumscribed with an associated lymphocytic infiltrate and contain epithelioid to spindled melanocytes with distinctive cytologic features including abundant cytoplasm and large nuclei containing prominent nucleoli.24 The cells are typically arranged in vertically oriented nests perpendicular to the epithelial surface. High-grade cytologic atypia is not present.

Molecular Features

Molecular data are limited but show that up to 50% of typical CN, like cutaneous nevi and melanoma, harbor BRAF V600E mutations.25,26 Data are lacking for conjunctival blue nevi despite knowledge of GNA11 and GNAQ mutations in cutaneous blue nevi and uveal melanoma.27,28 Deep penetrating nevi of the conjunctiva likely show a similar molecular profile to their cutaneous counterparts. In a recent study, all conjunctival DPNs showed positive immunohistochemical staining for BRAF V600E and evidence of β-catenin pathway activation (diffuse nuclear staining including in deep subepithelial melanocytes by immunohistochemistry).29

Clinical Management

Given the benign nature of most CN, the usual course of action consists of serial observation, slit lamp examination, and photographic documentation by an ophthalmologist.30 Benign nevi may vary in appearance over time, based on the hormonal status of the individual, with changes seen both in pregnancy and in adolescence. Changes in hormonal milieu may alter the amount of apparent pigment as well as the size of a benign nevus.31,32 Nevi that require excision are often located at the fornix, tarsal, or bulbar conjunctiva and show significant growth, appearance of neovascularization or nutrient vessels, inflammation, or increase or change in pigmentation. Additionally, nevi that recur after initial excision should be excised and examined histologically.29

PRIMARY ACQUIRED MELANOSIS/CONJUNCTIVAL MELANOCYTIC INTRAEPITHELIAL NEOPLASIA/CONJUNCTIVAL MELANOCYTIC INTRAEPITHELIAL LESIONS

Clinical Features

PAM is a group of nonnevoid intraepithelial melanocytic proliferations that accounts for 11% of conjunctival tumors and 21% of melanocytic lesions.8 PAM is mostly found in Caucasian adults in the sixth decade of life (mean patient age at time of diagnosis, 56 years; range, 15–90 years), although it has been demonstrated in African Americans, Hispanics, and Asians.15,30 The study by Shields et al demonstrated a slight female predominance in cases of PAM (62%). Curiously, the lesions are most commonly seen in patients with brown irides. Clinically PAM lesions are usually asymptomatic, unilateral, flat, noncystic lesions (Figure 3, A) that can be found throughout the conjunctiva, but are most commonly seen in the bulbar conjunctiva, with 57% located in the temporal quadrant. Although most commonly seen on the bulbar surface, lesions of the tarsal conjunctiva (Figure 3, B) or fornix are of particular concern. The pigmentation in PAM is heterogeneous and the size and pigmentation of the lesions may change over time.13 An extensive lesion (Figure 3, C) is considerable cause for concern, as most PAM lesions are focal, defined as less than or equal to 3 clock hours (Figure 4), with a mean size of 8 mm. Although complexion-associated melanosis, a benign process discussed previously under Benign Epithelial Hypermelanos of the Conjunctiva, can closely resemble PAM, complexion-associated melanosis (Figure 1, A) tends to present bilaterally and symmetrically in patients with dark complexion and is more commonly seen at the limbus, where it may form conjunctival microfolds.

Histologic Features

Historically, a biopsy representing a lesion with a clinical diagnosis of PAM is placed by the pathologist into 2 distinct histopathologic categories: PAM with atypia and PAM without atypia, which are clinically indistinguishable. The distinction between these 2 categories is essential as PAM with atypia has been shown to progress to invasive melanoma in 46% of cases.31 However, PAM without atypia is thought to have no malignant potential.12 Normal melanocytes, which contain minimal cytoplasm, contain a nucleus smaller than that of keratinocytes, which is typically round and slightly hyperchromatic without a visible nucleolus (Figure 5, A). PAM without atypia is characterized by a proliferation of small polyhedral melanocytes that lack cytomorphic atypia and are predominantly confined to the basilar layer of the epithelium.32 Historically, PAM without atypia has also included acquired hypermelanosis/hyperpigmentation without melanocytic hyperplasia, a condition some would argue should not be included within the realm of hyperplastic or neoplastic melanocytic lesions.9 PAM without atypia is histologically analogous to lentigo of the skin, which also does not harbor malignant potential.

The term primary acquired melanosis, although useful as a clinical term, is perhaps a misnomer in the cases of “primary acquired melanosis with atypia,” because these are thought to be neoplastic atypical melanocytic proliferations with a
potential for progression. It remains an important concept, however, because unlike the skin, where melanoma often arises from dysplastic nevi or de novo, most CMs arise from these precursor intraepithelial melanocytic proliferations. The term primary acquired melanosis is still most familiar to ophthalmologists. As a rule, ophthalmologists will not treat PAM without atypia and they will excise with margins, use cryotherapy, or give topical chemotherapy for PAM with atypia. In the authors' experience, the preferred treatment modality can depend on the degree of atypia. Clinicians will generally excise PAM with severe atypia, while PAM with mild atypia will be treated with topical agents or simply observed. However, these observations may be limited to our specific practice environment. The overall concept is similar to the evolution of lentigo maligna to lentigo maligna melanoma in situ to lentigo maligna melanoma in the skin.33,34 Indeed, given the high incidence of progression from PAM with atypia to melanoma, some pathologists (particularly dermatopathologists) have referred to this histologic pattern as melanoma in situ.35–37 The prior (7th) edition of the AJCC Cancer Staging Manual also referred to this pattern as melanoma in situ.38 However, many ophthalmic oncologists are reluctant to adopt the terminology despite the substantial risk of progression of such lesions to invasive melanoma, possibly owing to concern for the potential to alarm patients, given that noninvasive disease does not yet have the potential to give rise to metastases.

Given the inherent difficulty with classifying so many lesions as PAM with atypia, Sugiura et al37 subclassified this category into low- and high-risk groups, focusing on the importance of features of melanocytic proliferation to help determine the likelihood of a coexisting or developing melanoma (Table 1), confirming the strong association of epithelioid cytologic features with invasive melanoma.

Figure 3. Clinical appearance of primary acquired melanosis (PAM)/conjunctival melanocytic intraepithelial neoplasia (C-MIN)/conjunctival melanocytic intraepithelial lesions (CMILs). A, PAM/C-MIN/CMILs are typically asymptomatic, unilateral, flat, and noncystic with irregular borders. B, Involvement of the palpebral conjunctiva is a concerning clinical feature for PAM/C-MIN/CMILs. C, This PAM/C-MIN/CMIL shows a large area of involvement with heavy pigmentation, irregular borders, and extension into the cornea and is clinically concerning for melanoma.

Figure 4. Measuring pigmented lesions of the conjunctiva. Melanocytic lesions of the conjunctiva are measured in “clock hours” (diagram for the left eye shown here). This measurement is obtained by displaying the conjunctiva as a flat surface divided into 12 sections. The number of sections the lesion occupies is a surrogate for size. Our original illustration is modeled after Damato and Coupland35 (2008).
which had been previously identified in the original description of PAM.\textsuperscript{31} The presence of epithelioid cytology with vesicular nuclei and prominent nucleoli are the most important features in this designation. Pagetoid spread, an important architectural quality of cutaneous melanoma in situ, can be difficult to assess histologically in the thin epithelium of the conjunctiva; however, immunohistochemical markers, such as SOX-10, are a helpful adjunct. Of the lesions classified as low risk, 15\% progressed into invasive melanoma and none of the lesions metastasized. A much larger number of cases (94\%) were associated with invasive melanoma and metastases (25\%) in the high-risk group.

The recently formulated C-MIN classification system provides a semiquantitative measure for grading the severity of the lesions based on the pattern of horizontal spread, degree of vertical spread from the basal layer toward the surface, and the degree of histologic atypia, which includes cytoplasmic changes, nucleoli, and mitotic activity.\textsuperscript{39} A numerical score (0–10) is assigned from these criteria (Figure 5, A through H, and Figure 6, A through D; Table 2). Normal conjunctiva, like skin, shows a pattern of scattered dendritic, basal layer melanocytes (Figure 5, A and B). Although controversial, as primary hypermelanosis does not refer to a neoplastic proliferation of melanocytes, such lesions can be referred to as C-MIN 0 (Figure 5, C and D). C-MIN 1 is analogous to PAM without atypia, which is histologically characterized by an increased number of dendritic melanocytes that are confined to the junction of the epithelium with no significant pagetoid spread and with normal-sized nuclei (Figure 5, E and F). If any of the following characteristics are present, the lesion is regarded as atypical: pagetoid spread, epithelioid cytology consisting of increased cytoplasm equal to or beyond that seen in keratinocytes, nucleoli, mitoses, nest formation, or confluent growth (Figure 5, G and H, and Figure 6, A through D). C-MIN 2–3 is analogous to PAM with mild-moderate atypia, and C-MIN 4 is analogous to PAM with severe atypia. Scores above 5 are analogous to cutaneous melanoma in situ (Figure 6, C and D; Table 3).\textsuperscript{40} Critics of the C-MIN classification scheme point out that no formal studies to support its use based on outcome data have been performed (until quite recently), and that placing all melanocytic lesions into a single category may not be biologically accurate.\textsuperscript{41}

Table 1. Subclassification of Primary Acquired Melanosis (PAM) With Atypia According to Histologic Features\textsuperscript{a}

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<th>PAM With Low-Risk Features</th>
<th>PAM With High-Risk Features</th>
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<tr>
<td>Small to medium-sized melanocytes</td>
<td>Atypical architecture, such as nesting or dominant nonbasilar spread</td>
</tr>
<tr>
<td>High nuclear to cytoplasmic ratio</td>
<td>Melanocytes with various degrees of epithelioid features, abundant cytoplasm, vesicular nuclei, prominent nucleoli</td>
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<tr>
<td>Small to medium-sized hyperchromatic nuclei without nucleoli</td>
<td>Single cell lentiginous growth</td>
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\textsuperscript{a} Patients with high-risk features are more likely to have concurrent invasive melanoma and/or to develop melanoma from the PAM lesion. Data derived from Sugira et al.\textsuperscript{37}

Although the C-MIN classification system is another attempt to classify intraepithelial melanocytic proliferations of the conjunctiva and to use terminology that reflects the underlying pathobiology, it has not been widely adopted. Other authors have proposed descriptive categorization of these lesions, such as in the practice of dermatopathology, by using phraseology such as “intraepithelial melanocytic proliferation” or “atypical lentiginous melanocytic proliferation.” A mild, moderate, or severe grade of atypia is then given, based on a combination of cytologic atypia, nesting, epithelial consumption, pagetoid spread, and other architectural features. The most recent World Health Organization (WHO) classification scheme proposes a 2-tiered system of “conjunctival melanocytic intraepithelial lesions (C Mikhail),”\textsuperscript{42} with separation into low-grade CMIL (equivalent to PAM without atypia, PAM with mild atypia, or C-MIN 1-2; Figure 5, E through H) and high-grade CMIL (equivalent to PAM with moderate to severe atypia or C-MIN 3-5; Figure 6, A and B; Table 3).\textsuperscript{40} Although equivalencies among the proposed WHO system, PAM, and C-MIN were provided, formal criteria for each designation of CMIL were not outlined.

A recent retrospective, multi-institutional study compared the interobserver variability and diagnostic accuracy among the 3 major classification systems (PAM, C-MIN, and CMIL).\textsuperscript{43} All 3 systems were found to have high interobserver agreement with respect to final diagnosis among the 12 participating ophthalmic pathologists, who reviewed digitally scanned hematoxylin-eosin–stained slides only. Although poor diagnostic agreement was seen in low-grade lesions, a high accuracy of 81\% to 83\% for identifying lesions with a potential for recurrence was seen in each classification system. One notable observation was that the C-MIN classification system prolonged interpretation times and showed slightly higher interobserver variability, as would be expected for a more complex classification scheme.

In addition to evaluating these 3 classification systems, Milman et al\textsuperscript{44} proposed a simplified nomenclature in which hypermelanosis and low-grade CMIL are combined into a single “low grade” category, and high-grade CMIL and CM in situ are combined into a single “high grade” category (Table 3). The authors also provide proposed histologic descriptions of each WHO category, in which low-grade CMIL is described as “predominantly basilar melanocytic hyperplasia with mild atypia (dendritic or small polyhedral, nonpigmented melanocytes with irregular nuclear contours, inconspicuous nucleoli, and inconspicuous or scant cytoplasm)” and high-grade CMIL as “significant nonbasilar hyperplasia of atypical melanocytes involving <50\% or epithelium without nests or melanocytes with epithelioid morphology.” Melanoma in situ is described as “atypical melanocytes with nesting or >50\% full-thickness intraepithelial migration.”

It should be noted that junctional nevi can enter into the histologic differential diagnosis in many cases of C-MIN; however, caution should be taken in making a diagnosis of a junctional nevus in a patient outside of the first 2 decades of life, as these lesions are uncommon in adults. Additionally, the spread of the intraepithelial component far beyond the confines of the lateral border of the subepithelial component in a lesion regarded as a compound nevus should raise suspicion for evolving PAM/C-MIN/CMIL.
Figure 5. Normal conjunctival melanocytic density, conjunctival hypermelanosis, and primary acquired melanosis (PAM) without atypia or with mild atypia/conjunctival melanocytic intraepithelial neoplasia (C-MIN) 1-2/low-grade conjunctival melanocytic intraepithelial lesions (CMIILs). A and B, Normal conjunctiva showing scattered basally located dendritic melanocytes (arrows in A) with small, slightly hyperchromatic nuclei and scant cytoplasm, which shows retraction artifact at the border of the cell. Numerous dendrites of melanocytes whose nuclei are not in the plane of section can be seen on the Melan-A stain. C and D, Primary conjunctival hypermelanosis shows well-spaced dendritic melanocytes confined to the basilar layer of the epithelium with increased melanin pigment predominantly localized in epithelial cells. E and F, PAM without atypia (C-MIN 1) shows an
Abbreviation: PAM, primary acquired melanosis.

Molecular Features

Information regarding the molecular features of PAM/C-MIN/CMIL is limited. Interestingly, however, in contrast to benign nevi, in a small series of PAM/C-MIN/CMILs, BRAF mutations were not identified, suggesting the presence of alternative driver mutations in these proliferations. However, BRAF mutations have been detected in melanomas arising in association with PAM/C-MIN/CMIL. Recently, TERT promoter mutations were identified in a small subset of PAM with atypia (8%).

Clinical Management

Histologic diagnosis is essential in determining appropriate treatment. The initial evaluation begins with a careful slit lamp examination and a detailed map of the lesion. Size is critical here. If the initial lesion is small (less than 1 clock hour) and limited to the bulbar conjunctiva, it can be closely monitored. If it is between 1 and 2 clock hours the patient should be offered excision or close serial observations. If the size is greater than 2 clock hours, excision is typically recommended. Large tumors may require cryotherapy as an adjunctive treatment. If the lesion is very large or unresectable, map biopsies are typically performed to assess severity and extent of disease, with topical chemotherapy as an adjunctive treatment. The initial evaluation begins with a careful slit lamp examination and a detailed map of the lesion.15,29 Size is critical here. If the initial lesion is small (less than 1 clock hour) and limited to the bulbar conjunctiva, it can be closely monitored. If it is between 1 and 2 clock hours the patient should be offered excision or close serial observations. If the size is greater than 2 clock hours, excision is typically recommended. Large tumors may require cryotherapy as an adjunctive treatment.

CONJUNCTIVAL MELANOMA

Clinical Features

Conjunctival melanoma (CM) is extremely rare, accounting for only 1.6% of noncutaneous melanomas and less than 5% of “ocular” melanomas, most of which arise from the uveal tract. However, the incidence of CM, which is associated with UV light exposure, is increasing in the USA, Finland, and Sweden, especially in Caucasian men (295% increase during a 27-year period). A recent study revealed that most patients with CM (85%) have a Fitzpatrick skin type (FST) of I or II, a trend comparable to that observed in cutaneous melanoma. CM is most common in the sixth decade of life (mean age at diagnosis, 62 years), with a slight male predominance (59%). Older patients tend to present with more extensive disease and have a higher risk for both loss of visual acuity and local recurrence. Although cases have been reported in children, this occurrence is exceedingly rare. CM most commonly arises in association with PAM/C-MIN/CMIL. De novo CM is less common, and CM arising in association with a nevus is uncommon.

At the time of presentation of CM, 98% of patients are asymptomatic. The most frequent symptoms include a visible spot, a lump, ocular irritation, and pain. In one large series, all cases of CM were unilateral, usually in the bulbar region (92%; Figure 7, A and B). There is a slight predominance of CM in the lateral quadrant (63%), followed by the inferior (22%), medial (17%), and superior (16%) quadrants. A minority of cases (3%) are described as diffuse. Most CMs extend past 2 clock hours (88%; Figure 7, C and D) and 61% touch the limbus. Most lesions are melanotic (68%); however, other hues are observed, such as red, yellow, or a combination of brown and yellow. Tumor feeder vessels are present in a minority of cases (39%).

Recurrences of CM are frequent. The mean time between the first and second recurrence is approximately 15 months; 26% of recurrences are detected at 5 years follow-up. Many factors are correlated with recurrence and include parameters associated with anatomic location such as CM not touching the limbus, melanoma more than 2 mm from the limbus, PAM/C-MIN/CMIL at the 7 to 9 clock hours, and melanoma located in the superior quadrant. Other factors include the presence of red coloration, involvement of the lateral and base margins at the time of excision, thickness greater than or equal to 2 mm, or corneal/globe involvement.

A minority of patients with CM develop metastases, approximately 18% in one large series. Of these, a third developed metastatic disease 15 years after their initial diagnosis. Location of the metastasis is most commonly a “preauricular” or parotid lymph node, but distant metastases have been observed in sites such as lung, brain, and liver. The mortality rate of CM is low, around 8%, and survival rates are 93% at 5 years and 87% at 10 years.

Table 2. Scoring System for Conjunctival Melanocytic Intraepithelial Neoplasia (C-MIN)a

<table>
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<tr>
<th>Growth pattern</th>
<th>Nil</th>
<th>Basal</th>
<th>Scattered pagetoid cells</th>
<th>Isolated nests</th>
<th>Confluent nests</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Choose one)</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Vertical (pagetoid) spread</td>
<td>Nil</td>
<td>&lt;50%</td>
<td>50%-90%</td>
<td>&gt;90%</td>
<td></td>
</tr>
<tr>
<td>(Choose one)</td>
<td>0</td>
<td></td>
<td>2</td>
<td>3</td>
<td>___/3</td>
</tr>
<tr>
<td>Melanocytic atypia</td>
<td>Nucleus/cytoplasm &lt; basal squamous cells</td>
<td>Nucleus ≥ basal squamous cells</td>
<td>Cytoplasm ≥ basal squamous cells</td>
<td>Nucleoli and/or mitoses</td>
<td>___/3</td>
</tr>
<tr>
<td>(Add all findings present)</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>___/10</td>
</tr>
</tbody>
</table>

Abbreviation: PAM, primary acquired melanosis.

a The maximum score is 10. A score of 1 is equivalent to a diagnosis of PAM without atypia; a score of 2, PAM with mild atypia; a score of 3, PAM with moderate atypia; a score of 4-5, PAM with severe atypia; and a score of >5, melanoma in situ. Data derived from Damato and Coupland (2006).
Several factors are associated with a poor prognosis (Table 4). The American Joint Committee on Cancer (AJCC) tumor, lymph node, and metastasis (TNM) staging system, which is determined clinically and pathologically, predicts the prognosis of CMs, with higher T-category tumors corresponding to a greater risk of adverse outcome. Surgical technique may also play a role in outcome.

**Histologic Features**

As most CMs arise from a junctional precursor lesion, the presence of a high-grade intraepithelial melanocytic proliferation overlying a subepithelial component should arouse suspicion for invasive melanoma and prompt careful evaluation. Several architectural features are more common in CM than in other melanocytic lesions and can be useful in diagnosing CM. These features include pagetoid spread of the intraepithelial component (Figure 8, A), disruption of the substantia propria by vertical extension of the intraepithelial component (Figure 8, B), inflammation at the base of the lesion (Figure 8, C), and an absence of maturation of the subepithelial component (Figure 8, B). Evaluation for asymmetry at low power can be a useful discriminator, as well as the presence of expansile/nodular growth (Figure 7, B). Often, CMs are composed of highly atypical melanocytes with nuclear atypia, vesicular chromatin, and prominent nucleoli (Figure 8, D). Spindle cells, large epithelioid cells, balloon cells, and small polyhedral cells can also be seen. As in cutaneous melanoma, cases of CM that histopathologically mimic nevi with a nested invasive component also occur (Figure 8, E and F). A number of factors are associated with poor prognosis and include tumor thickness greater than 2.0 mm, tumor location (nonbulbar), mitotic activity greater than 1 per mm², ulceration, vascular invasion, epithelioid cell type, microsatellitosis, and positive margin status (Table 4). Conjunctival melanoma may also
involve the epithelium of the cornea and invade into its stroma, or extend onto the eyelid skin.

Like some melanomas arising in the skin, a subset of CMs is associated with loss of p16 immunohistochemical expression. However, this finding is not entirely specific for malignancy. Interestingly, p16 staining may correlate with depth of invasion. In one study, "superficial melanomas," defined as lesions with a vertical infiltration of less than 2 mm, had higher p16 levels than melanomas infiltrating to a deeper level in the dermis. This suggests that p16 could potentially be used as a prognostic indicator in this setting; however, this finding was not replicated in a more recent study. Additionally, in this limited series, loss of p16 expression was not associated with copy number loss or mutation of CDKN2A.

Immunohistochemistry has been shown to be a useful adjunct in the evaluation of conjunctival melanocytic lesions. Utilization of markers such as SOX-10 and Melan-A/MART-1 can be used to assist in appreciation of architectural features of a conjunctival melanocytic prolifer-

Table 3. Comparison of Primary Acquired Melanosis Classification to the Conjunctival Melanocytic Intraepithelial Neoplasia Classification Developed by Damato and Coupland, the Proposed World Health Organization (WHO) Classification Scheme, and Simplified WHO Terminology Proposed by Milman et al

<table>
<thead>
<tr>
<th>PAM Classification (Hypermelanosis and Conjunctival Melanoma In Situ Added)</th>
<th>C-MIN Score (of 10)</th>
<th>Proposed WHO Terminology</th>
<th>Simplified WHO Terminology Proposed by Milman et al (2021)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAM without atypia (hypermelanosis only)</td>
<td>0</td>
<td>Hypermelanosis only</td>
<td>Low grade</td>
</tr>
<tr>
<td>PAM without atypia (hyperplasia of melanocytes without atypia)</td>
<td>1</td>
<td>Low-grade CMIL</td>
<td></td>
</tr>
<tr>
<td>PAM with mild atypia</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAM with moderate atypia (no epithelioid cytomorphology)</td>
<td>3</td>
<td>High-grade CMIL</td>
<td>High grade</td>
</tr>
<tr>
<td>PAM with severe atypia (epithelioid cytomorphology but not full-thickness)</td>
<td>4-5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAM with severe atypia (conjunctival melanoma in situ)</td>
<td>≥5</td>
<td>Conjunctival melanoma in situ</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CMIL, conjunctival melanocytic intraepithelial lesion; C-MIN, conjunctival melanocytic intraepithelial neoplasia; PAM, primary acquired melanosis.

Figure 7. Clinical photographs of conjunctival melanoma (CM). Although some CMs are focal (A and B), most CMs are relatively large and extend past 2 clock hours (C and D). CMs are typically brown in coloration, although they can also contain red, yellow, or a combination of these colors.
Figure 8. Histologic images of conjunctival melanoma (CM). A, Intraepithelial component of an example of CM demonstrating prominent pagetoid spread, irregular nest formation, and cytologic atypia in the form of nuclear hyperchromasia. B, Invasive melanoma showing expansion of the substantia propria with large sheets of melanocytes without evidence of maturation. C, Inflammation at the base of the lesion is often seen in CM. D, A high-power view demonstrates cytologic atypia with enlarged nuclei and prominent nucleoli with numerous mitotic figures. E, Conjunctival melanoma with a nested invasive component, which shows a lack of maturation with descent into the substantia propria. The junctional component meets criteria for melanoma in situ with confluent growth and prominent pagetoid spread of melanocytes. F, Although the subepithelial component is
Conjunctival Melanocytic Lesions

Molecular Features

Molecular diagnostic studies can be useful adjuncts in discriminating between benign and malignant melanocytic conjunctival lesions, especially in cases with borderline histology. Molecular aberrations, including copy number variations, are similar to those of cutaneous and mucosal melanoma, and are entirely distinct from those of uveal melanoma. Fluorescence in situ hybridization (FISH) may identify copy number gain of the RREB1 (6p25), MYB (6q23), CCND1 (11q13), or MYC (8q24) genes as well as copy number loss of CDKN2A (9p21). However, the technique may lack sensitivity in detecting CM.

Array comparative genomic hybridization can provide a genome-wide survey of copy number alterations. However, as in cutaneous melanocytic lesions, when interpreting FISH or other copy number data, one must remain cautious and not disregard the histopathologic impression, which continues to be the gold standard.

With respect to genetic mutations, cases of CM typically show a cytosine to thymine mutation signature, characteristic of UV light–induced DNA damage. Up to 50% of CMs harbor BRAF V600E mutations; furthermore, the presence of a BRAF mutation, which occurs less commonly in melanomas arising in PAM/C-MIN/CMIL, may be associated with a higher rate of distant metastasis. Like cutaneous melanomas, in the absence of a BRAF mutation, common aberrations are seen in NRAS and NF1, also part of the mitogen-activated protein kinase (MAPK) pathway, which tend to be mutually exclusive.

At least 4 subgroups: BRAF mutated, RAS mutated, NF1 mutated, and triple wild-type. One recent study observed CCND1 amplification as well as CIC mutation in a triple wild-type tumor. CIC encodes a transcription factor that regulates the MAPK pathway.

Clinical Management

The pathologist should be aware of the differences in treatment between cutaneous and conjunctival melanomas. Generally, the treatment of CM largely depends on the size of the lesion. Primary excision is best performed by a surgeon experienced in conjunctival melanocytic lesions, as primary surgery by a nonexpert is a risk factor for recurrence. Some authors advocate that the lesion should be fully excised en bloc without touching the tumor directly (no touch technique) and only handling surrounding normal tissue.

The goal with removing the melanoma is to obtain wide clear margins (3–4 mm). Cryotherapy of the adjacent remaining conjunctival tissue is applied to eradicate any potential subclinical disease. The size of the deficit created by the excision is assessed once the tumor has been excised. Primary closure of Tenon fascia and then conjunctiva is performed for reconstruction. An amniotic membrane can be used to close the surgical site if the defect is too large for primary closure. Superthin partial sclerectomy of the scleral base and alcohol epitheliectomy of the cornea allow for tumor-free margins. One observational study of 150 patients reported that cases managed with excisional biopsy using the no-touch technique plus alcohol corneal epitheliectomy and supplemental cryotherapy have a lower risk of tumor recurrence, metastasis, or death than those treated with excisional biopsy alone.

Topical chemotherapy with mitomycin-C is widely used as adjunct therapy if intraepithelial disease is present at margins after surgery; however, if the margins are positive for invasive melanoma, reexcision may be optimal. Punctal occlusion may provide protection against complications such as punctal stenosis or damage to the nasopharyngeal epitelium. Topical chemotherapy can be applied to the entire ocular surface, to treat diffuse disease or large areas of disease that cannot be surgically removed, particularly for purely in situ lesions. If the tumor is large and locally advanced, enucleation (removal of the eye) or exenteration (removal of the orbit) and radiation may be necessary.

The use of sentinel lymph node biopsy remains controversial for the staging and management of CM, but is emerging.

Table 4. Poor Prognostic Indicators of Conjunctival Melanoma

<table>
<thead>
<tr>
<th>Tumor thickness &gt;2.0 mm</th>
<th>Tumor location (nonbulbar)</th>
<th>Mitotic activity greater than 1/mm³</th>
<th>Ulceration</th>
<th>Vascular invasion</th>
<th>Epithelioid cell type</th>
<th>Microsatellitosis</th>
<th>Margin status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive sentinel lymph node biopsy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a Data derived from Folberg and McLean,12 1986; Shields et al,48 2011; Tuomaala et al,57 2002; and Esmaeli et al,81 2019.

Compared to cutaneous melanomas, conjunctival lesions, when interpreting FISH or other copy number data, one must remain cautious and not disregard the histopathologic impression, which continues to be the gold standard.67 As in cutaneous melanoma, the mutational status of these 3 genes allows classification into 4 subgroups: BRAF mutated, RAS mutated, NF1 mutated, and triple wild-type. One recent study observed CCND1 amplification as well as CIC mutation in a triple wild-type tumor. CIC encodes a transcription factor that regulates the MAPK pathway.

HMB-45 expression (Figure 8, G) has been shown to be predictive of melanoma. An increased Ki-67 proliferation index.

Associated with a higher rate of distant metastasis. Like cutaneous melanomas, in the absence of a BRAF mutation, common aberrations are seen in NRAS and NF1, also part of the mitogen-activated protein kinase (MAPK) pathway, which tend to be mutually exclusive.

As in cutaneous melanoma, the mutational status of these 3 genes allows classification into 4 subgroups: BRAF mutated, RAS mutated, NF1 mutated, and triple wild-type. One recent study observed CCND1 amplification as well as CIC mutation in a triple wild-type tumor. CIC encodes a transcription factor that regulates the MAPK pathway. TERT promoter mutations appear to be frequent in CM, with one study reporting them in 41% of the cases studied.

Mutations in epigenetic regulatory genes, such as ASXL1, ATRX, and TET2, have been reported, as well as aberrations in genes that encode receptor tyrosine kinases, such as EGFR and ALK. In contrast to melanomas at mucosal sites that are not sun exposed, CM rarely contains clinically actionable mutations in KIT.
as a possible important prognostic indicator. Sentinel lymph node biopsies, routinely used in cutaneous melanoma, can help to identify micrometastases and macrometastases, providing important prognostic information and identifying patients who are more likely to benefit from targeted or immune-modulatory therapies. Notably, although the technique appears to be both safe and effective, no randomized studies have been conducted with respect to the use of sentinel lymph node biopsy in CM. However, 2 recent and relatively large observational studies (together totaling 53 patients), using a depth of invasion cutoff of at least 1 mm or more than 2 mm to determine eligibility for the procedure, showed the presence of micrometastasis in 11% to 13% of patients. In one study, the presence of sentinel node metastasis was correlated with higher depth of invasion, increased mitotic rate, and the presence of lymphovascular invasion, perineural invasion, or ulceration. Interestingly, tumor location, which is an important determinant of T-category according to the current AJCC criteria, was not significantly associated with a positive sentinel lymph node biopsy. A third recent study including 88 total patients confirmed depth of invasion and ulceration as the most important factors predicting lymph node involvement, as well as distant metastasis and disease-related death. Additionally, in this study, the risk of distant metastasis and disease-related death did not differ between T1 (bulbar) and T2 (nonbulbar) tumors, or between T2c,d (caruncular) and T1-T2a,b (non-caruncular) tumors. Although limited in sample size, this study showed a higher risk of distant metastasis and death from disease in patients with a positive sentinel lymph node biopsy finding. However, although sentinel lymph node biopsy may provide important prognostic information, the technique has not been shown to prevent future distant metastases or prolong survival in CM.

Like the treatment of primary CM, recurrences of CM are treated with a variety of techniques either alone or in combination with complete resection, radiotherapy, or in advanced cases, exenteration. While surgical resection, cryotherapy, and topical chemotherapy are the mainstay of CM treatment now, recent discovery of genetic mutations in CM, specifically within BRAF, which has been cited in 22% to 40% in CM, may offer potential therapeutic targets.

**SUMMARY**

As in the skin, a range of melanocytic lesions affect the conjunctiva and span from complexion-related melanosis to benign nevi to melanoma. These lesions can be triaged on their clinical appearance, but in some cases require histologic diagnosis. A number of important differences exist in the classification of nevi and premalignant melanocytic proliferations. Understanding the various classification systems used for conjunctival melanocytic lesions is of paramount importance for effective communication between providers and for ensuring appropriate clinical follow-up and treatment of patients. Interestingly, however, despite historical differences in classification and staging, CMs are genetically similar to their cutaneous counterparts, showing similar oncogenic driver mutations and copy number alteration profiles. This provides rationale for the use of targeted systemic agents such as BRAF and other MAPK pathway protein inhibitors, and allows for the utilization of powerful new diagnostic tools, such as FISH and array comparative genomic hybridization, to aid in the classification of histologically borderline tumors.

**References**