Indeterminate Melanocytic Proliferations of the Conjunctiva

Hans E. Grossniklaus, MD; Curtis E. Margo, MD, MPH; Alvin R. Solomon, MD

Objective: To test the hypothesis that a subset of conjunctival melanocytic proliferations exists that cannot be reproducibly classified as benign, malignant, or indeterminate.

Methods: Three groups of excisional biopsy specimens of conjunctival melanocytic proliferations were evaluated by a panel of 5 ophthalmic pathologists. These groups included lesions that we considered to represent benign (group 1 [n = 5]), malignant (group 2 [n = 5]), and indeterminate melanocytic proliferations (group 3 [n = 5]). The panel classified the same sections in all 3 groups in a randomized, masked fashion, first without and then with a clinical history of patient age, sex, and race. The κ statistic was used to quantify the degree of agreement among observers.

Results: There was strong concordance among the panel members for both group 1 (benign [κ = 0.76]) and group 2 (malignant [κ = 0.70]) melanocytic proliferations. There was no concordance of the panel for group 3 (indeterminate) lesions (κ = −0.045). The concordance for groups 1 and 2 and lack of concordance for group 3 lesions were independent of knowledge of clinical history of age, sex, and race.

Conclusion: A subset of melanocytic proliferations of the conjunctiva exists that cannot be reproducibly classified by pathologists as benign, malignant, or indeterminate.


THE CLASSIFICATION of melanocytic proliferations of the conjunctiva is a process in which every lesion is considered to be benign, malignant, or indeterminate. The conceptual framework for this system is based on the assumption that the histopathologic diagnosis of melanocytic proliferations is both accurate and precise (reproducible). Although the histopathologic features of benign and malignant conjunctival melanocytic proliferations are well defined, a subset of indeterminate proliferations exists that cannot be easily classified.

See also page 1121

This group of indeterminate proliferations has features of both benign and malignant neoplasms. When these lesions are reviewed and interpreted by different pathologists, there is no consensus. The problems encountered when experienced pathologists cannot agree on the biologic potential of a melanocytic lesion are considerable because the prognosis and clinical management differ greatly between the extremes of benign and malignant. We identified a group of indeterminate conjunctival melanocytic proliferations that had features of both benign and malignant neoplasms. We designed an experiment to test the hypothesis that pathologists cannot reproducibly classify a subset of conjunctival melanocytic proliferations as benign, malignant, or indeterminate.

RESULTS

The age, sex, and race of the patients in the 3 groups are summarized in Table 1. All cases were unilateral. The average ages of the patients in groups 1, 2, and 3 were 30, 58, and 17 years, respectively. There were more women than men in all 3 groups. The classification of the panel members is summarized in Table 2. There were no group 1 lesions classified as malignant and no group 2 lesions classified as benign by the panel. If the interpretation was changed after the history was revealed, the lesions from patients aged 40 years and younger were all downgraded and for patients aged 41 years or older were all upgraded. There was strong concordance among the panel members for prehistory and posthistory group 1 (κ, 0.58 and 0.76, respectively) and group 2 (κ, 0.70 and 0.70, respectively) lesions. There
was no concordance for prehistory and posthistory group 3 lesions (κ, −0.075 and −0.045, respectively). There was no statistically significant difference between prehistory and posthistory interpretation of the cases.

**COMMENT**

Conjunctival melanoma, although rare, is the second most common conjunctival malignant neoplasm, after squamous cell carcinoma. Conjunctival melanoma, which is associated with a mortality rate of approximately 40%, is a higher-grade malignant neoplasm than conjunctival squamous cell carcinoma, which rarely metastasizes. Therefore, melanoma is the most common high-grade malignant neoplasm of the conjunctiva, and accurate diagnosis is important for treatment and prognosis.

Concepts regarding the pathogenesis of conjunctival melanoma have changed in recent years. Early theories proposed that conjunctival melanoma arose de novo from preexisting nevi with a junctional component or from primary acquired melanosis (PAM). It is now postulated that approximately 75% of conjunctival melanomas arise from PAM and the remainder rapidly develop without antecedent PAM, roughly analogous to cutaneous nodular melanoma. Primary acquired melanosis with atypia has characteristic histologic features, although in some instances, PAM with atypia may be histologically indistinguishable from junctional nevus. In these instances, clinical information regarding patient age, sex, race, and laterality is helpful in distinguishing PAM with atypia from junctional nevus.

Studies regarding conjunctival melanoma, its precursors, and benign melanocytic lesions have been mostly limited to adult patients. The largest study to date of conjunctival melanocytic proliferations in children included 71 patients. Three patients had PAM, 3 had melanoma, and the remainder had nevi. It is well known that disease processes with similar histopathologic features may behave differently in children vs adults. In at least one published case, it was impossible for a group of experienced ophthalmic pathologists to determine whether a conjunctival melanocytic proliferation in a child was benign or malignant. In that case, as in other cases that we are aware of, the initial histologic classification of the lesion was melanoma, and the classification was downgraded to nevus with atypia when the patient’s age was known. We have noted a variety of outcomes in these difficult cases, ranging from cure after a biopsy to progression to metastatic melanoma and death (A. Proia, MD, PhD, unpublished data, May 9, 1999).

The benign conjunctival melanocytic proliferations (group 1) in this study included what we interpreted to represent compound nevi, the most common type of conjunctival nevus. All group 1 lesions displayed tightly coherent junctional and subepithelial nests of cells with maturation from the surface to the base of the lesion (Figure 1). There were epithelial cystic invaginations present in all group 1 lesions, and none of

**STATISTICAL ANALYSIS**

The κ coefficient was calculated as observed agreement among the 3 possible diagnostic categories (variables) minus chance agreement divided by 1 minus chance agreement. When there is perfect agreement among measured variables, the κ coefficient is 1. For purely chance agreement (ie, random allocation), the κ coefficient is 0. A negative κ coefficient value means that the lack of agreement was worse than anticipated by random allocation of variables.

**MATERIALS AND METHODS**

**STUDY DESIGN**

To test the hypothesis that there is a subset of indeterminate conjunctival melanocytic proliferations for which consistent histologic classification is impossible, we identified 5 cases each of conjunctival nevus (group 1), melanoma (group 2), and indeterminate melanocytic proliferation (group 3). We agreed on the classification of each case prior to inclusion in the study. A detailed discussion of the histopathologic features of group 1, 2, and 3 lesions is provided in the “Comment” section of this article. A slide from each case was evaluated in a randomized masked fashion by 5 ophthalmic pathologists asked to classify the lesions as benign, malignant, or indeterminate before and after the clinical information was provided.

Each member of the panel of ophthalmic pathologists was board certified in anatomic pathology and 2 panel members were also board certified in ophthalmology. The panel members were actively practicing ophthalmic pathology from medical centers in different geographic regions in the United States at the time of the study. Each panel member reviewed the same slides of the group 1, 2, and 3 lesions.

**STATISTICAL ANALYSIS**

The κ coefficient was calculated as observed agreement among the 3 possible diagnostic categories (variables) minus chance agreement divided by 1 minus chance agreement. When there is perfect agreement among measured variables, the κ coefficient is 1. For purely chance agreement (ie, random allocation), the κ coefficient is 0. A negative κ coefficient value means that the lack of agreement was worse than anticipated by random allocation of variables.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age, y</th>
<th>Sex/Race</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>43</td>
<td>Male/white</td>
</tr>
<tr>
<td>2</td>
<td>31</td>
<td>Female/white</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>Female/white</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>Female/white</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>Male/white</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>42</td>
<td>Female/white</td>
</tr>
<tr>
<td>7</td>
<td>47</td>
<td>Male/white</td>
</tr>
<tr>
<td>8</td>
<td>80</td>
<td>Male/white</td>
</tr>
<tr>
<td>9</td>
<td>45</td>
<td>Female/white</td>
</tr>
<tr>
<td>10</td>
<td>76</td>
<td>Female/white</td>
</tr>
<tr>
<td>Group 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>21</td>
<td>Female/black</td>
</tr>
<tr>
<td>12</td>
<td>2</td>
<td>Female/white</td>
</tr>
<tr>
<td>13</td>
<td>9</td>
<td>Female/white</td>
</tr>
<tr>
<td>14</td>
<td>46</td>
<td>Female/white</td>
</tr>
<tr>
<td>15</td>
<td>8</td>
<td>Male/Indian</td>
</tr>
</tbody>
</table>

*All cases were unilateral.*
these lesions displayed cellular atypia. The malignant conjunctival melanocytic proliferations (group 2) were all considered by us to represent melanoma. All group 2 lesions contained loosely coherent pleomorphic cells and had marked junctional activity and no maturation from the surface to the base of the lesion (Figure 2). There was mitotic activity in group 2 lesions, and all of these lesions clearly invaded the substantia propria. The indeterminate conjunctival melanocytic proliferations (group 3) were all cases in which we could not agree if the lesion represented PAM, nevus, melanoma, or any combination of the 3. All group 3 cases had junctional activity of the melanocytic proliferation (Figures 3, 4, and 5). These lesions exhibited extension of pleomorphic cells into the substantia propria with some degree of loss of maturation from the surface to the base of the lesion (Figure 5). The group 3 lesions also exhibited intraepithelial extension to near full epithelial thickness in areas. All group 3 lesions were located on the bulbar conjunctiva and displayed varying degrees of pigmentation (Figures 3, 4, and 5).

The mitotic count per 40 high-power microscopic fields was 0 cases for group 1, 2 of 2 cases for group 2, and 1 of 1 case for group 3. Evaluation of mitotic activity is useful, but it is not an absolute criterion for benign vs malignant neoplasm and must be considered along with other parameters, including overall configuration of the lesion and cytologic features. Addi-
tionally, immunostaining for melanocytic activity and proliferation with HMB-45,\textsuperscript{14} evaluation of nucleolar organizer regions,\textsuperscript{15} and electron microscopic examination\textsuperscript{16} seem to elicit borderline findings in indeterminate conjunctival melanocytic proliferations, just as the histologic findings are borderline.

At least 20 different types of benign melanocytic proliferations of the conjunctiva have been described.\textsuperscript{1} Several of these entities may be cytologically worrisome, such as inflamed nevus and Spitz nevus.\textsuperscript{1} Unusual variants of conjunctival nevi, including epithelioid nevus, spindle nevus, and dysplastic nevus, have been reported.\textsuperscript{17} Our group

---

**Figure 2.** Case 10. Malignant conjunctival melanocytic proliferation (malignant melanoma) (group 2, case 10). Left, This lesion consists of a loosely coherent sheet of melanocytes without maturation from the surface to the base (hematoxylin-eosin, original magnification ×25). Right, There is marked junctional activity of pleomorphic melanocytes (hematoxylin-eosin, original magnification ×63).

**Figure 3.** Case 11. Indeterminate conjunctival melanocytic proliferation (group 3, case 11). Left, This variably pigmented lesion (arrow) is present at the limbus of this 21-year-old African American woman. Right, The lesion consists of loosely coherent junctional nests of pleomorphic melanocytes (arrow) with horizontal extension (arrowheads). There are epithelial inclusion cysts (*) and a lymphocytic infiltrate (L) in the substantia propria. This lesion recurred and the recurrence was classified as a malignant melanoma (hematoxylin-eosin, original magnification ×63).

**Figure 4.** Case 12. Indeterminate conjunctival melanocytic proliferation (group 3, case 12). Left, This amelanotic lesion (arrow) is present in the bulbar conjunctiva of this 2-year-old white girl. Right, The lesion is composed of loosely coherent junctional nests of cells (arrow) with horizontal extension (arrowheads) and invasion into the substantia propria (open arrows). Epithelial inclusion cysts (*) are present (hematoxylin-eosin, original magnification ×63).
3 indeterminate lesions did not display the histologic features of Spitz, epithelioid, spindle cell, or dysplastic nevus. Although occasional lymphocytes were present in some of our indeterminate lesions, this was a minor feature, and we felt that none of these warranted a diagnosis of inflamed nevus. On the contrary, the lymphocytes in these indeterminate cases were often organized in patches or bands at the base of the lesion, a feature more suggestive of melanoma than nevus.2

The concept that melanoma arises in PAM with atypia that may be associated with nevi warrants consideration with regard to our indeterminate category. Primary acquired melanosis with atypia implies cytologic atypia and atypical patterns of proliferation of melanocytes.2 Atypical cell types include small epithelioid, spindle, dendritic, and large epithelioid and atypical patterns, such as basilar hyperplasia, basilar nests, intraepithelial nests, pagetoid spread, and complete epithelial replacement.2 There are often combinations of these cytologic features and growth patterns in a single lesion. When these pleomorphic cells invade the substantia propria, the lesion is classified as melanoma. Primary acquired melanosis with atypia may be seen in association with nevi,16 and what was formerly interpreted to represent melanoma arising in nevus is now postulated to represent melanoma arising in PAM associated with nevus.5

Primary acquired melanosis is typically unilateral, arises in middle-aged to elderly white patients, and is more common in women than men.2,10 All of our indeterminate (group 3) lesions were unilateral and all but one occurred in patients aged 21 years or younger. Group 3 lesions contained small and large epithelioid cells and polyhedral cells arranged in junctional and intraepithelial nests with rare pagetoid cells. There was no evidence of basilar hyperplasia or complete epithelial replacement in these lesions. Although these features are consistent with PAM with atypia, atypical cells in the substantia propria in all group 3 lesions showed many indications of melanoma (Figure 5). The degree of pleomorphism and lack of cohesiveness were not sufficient, in our opinion, to warrant an unequivocal diagnosis of melanoma. Helpful features in making the histologic diagnosis of melanoma are junctional activity, loss of maturation from the surface to the base of the lesion, invasion into the substantia propria, discohesiveness, and loss of symmetry of the entire lesion. We cannot exclude the possibility that these lesions contain PAM with atypia or a nevus with atypia, although we also cannot exclude the possibility that these lesions are melanoma. Hence, the term indeterminate melanocytic proliferation is used.

All group 3 patients were followed up for at least 2 years. The lesion was incompletely excised in patients 11 and 12 and completely excised in patients 13 through 15. The lesion recurred 3 times in patient 11 and was unequivocally classified as melanoma on the third recurrence. The lesion did not recur in patients 12 through 15. Therefore, the natural history of indeterminate melanocytic proliferation of the conjunctiva is unknown, the biologic behavior is unpredictable, and possible outcomes range from regression after incomplete removal to progression to melanoma.

There are other case reports of indeterminate conjunctival melanocytic proliferation, mainly occurring in children.3,12 This entity was recognized by Margo and coworkers5 in a 13-year-old girl. The histologic features in that case were very similar to our 5 indeterminate lesions. McDonnell and coworkers12 noted in their series of 71 conjunctival melanocytic proliferations in patients aged 20 years or younger that the original diagnosis in 6 cases was atypical nevus. Their review of those 6 cases showed that 1 case was a Spitz nevus, 1 was a cellular blue nevus, and 1 had noncohesiveness of the junctional nests.12 We would probably classify the latter case as indeterminate melanocytic proliferation. The authors found no worrisome features in the 3 remaining cases.12 The 3 conjunctival melanomas in the series by McDonnell and coworkers12 had histologic features unequivocal for melanoma. The case described by Margo et al5 and at least one of the cases in the study by McDonnell and coworkers would fit our criteria for indeterminate melanocytic proliferation.

The fact that there was a discrepancy between the original diagnosis and the review diagnosis in at least 5 patients in the study by McDonnell et al5 is not surprising. Farmer and coworkers20 found only moderate interobserver agreement in a masked study of 37 classic cutaneous nevi and melanomas. Our series showed strong
interobserver prehistory and posthistory concordance for classic conjunctival nevi (group 1: $\kappa$, 0.58 and 0.76, respectively) and classic conjunctival melanoma (group 2: $\kappa$, 0.70 and 0.70, respectively). There was absolutely no prehistory and posthistory concordance in our indeterminate category (group 3: $\kappa$ -0.075 and -0.045, respectively). In fact, the negative $\kappa$ statistics for group 3 mean that there was less concordance than expected if the observers randomly selected a diagnosis of benign, malignant, or indeterminate. Knowledge of the clinical history did not affect this lack of concordance. We believe the panel represented a group of well-trained, experienced ophthalmic pathologists who are likely to encounter this spectrum of lesions.

A practical consideration is how to treat patients with these indeterminate lesions. Since the biologic behavior is uncertain and a given indeterminate lesion may represent a developing melanoma, complete surgical removal is warranted. There are no good data regarding the adequacy of surgical margins of excision for conjunctival melanocytic proliferations. If the clinical suspicion is high for melanoma, we recommend a 3- to 5-mm margin. In children and young persons with hormonal changes, a 1-mm margin may be adequate. These recommendations must be tempered with clinical information, including the health of the patient and extent of the lesion. Alternative therapies, such as cryotherapy, may be considered in selected instances.

A subset of conjunctival melanocytic proliferations exists that is impossible for experienced, qualified ophthalmic pathologists to consistently classify as benign or malignant and thus predict biologic behavior. A diagnosis of indeterminate or borderline melanocytic proliferation is appropriate for this entity because it is impossible to pigeonhole these lesions into existing dichotomous classification schemes.

Recognizing the existence of an indeterminate group of melanocytic lesions has conceptual and practical importance. First, without the indeterminate category, lesions that fall into this group will be regularly misdiagnosed. Because there was no agreement among pathologists on the diagnosis of cases in the indeterminate subset, the assignment of a diagnosis of benign or malignant in a dichotomous system would be random. Second, the recognition of the indeterminate group will require ocular oncologists to develop appropriate protocols for management. Since the consequences of undertreating a malignant tumor are potentially worse than overtreating a benign one, protocols for tumor removal or destruction and follow-up appropriate for a tumor of unknown biologic potential will need to be developed. Third, the indeterminate category is, in effect, a confession that histopathologic classification is not absolute and that improved methods of predicting biologic behavior of tumors are needed. While most pathologists appreciate the limitations of histopathologic interpretation, many clinicians have the mistaken belief that the process is absolute. An indeterminate category of melanocytic lesions should help improve communication with clinicians because it conveys the lack of certainty in diagnosis more clearly than terms such as atypical nevus or borderline melanoma.

**CONCLUSIONS**

A subset of conjunctival melanocytic proliferations exists that is impossible for experienced, qualified ophthalmic pathologists to consistently classify as benign or malignant and thus predict biologic behavior. A diagnosis of indeterminate or borderline melanocytic proliferation is appropriate for this entity because it is impossible to pigeonhole these lesions into existing dichotomous classification schemes.

Recognizing the existence of an indeterminate group of melanocytic lesions has conceptual and practical importance. First, without the indeterminate category, lesions that fall into this group will be regularly misdiagnosed. Because there was no agreement among pathologists on the diagnosis of cases in the indeterminate subset, the assignment of a diagnosis of benign or malignant in a dichotomous system would be random. Second, the recognition of the indeterminate group will require ocular oncologists to develop appropriate protocols for management. Since the consequences of undertreating a malignant tumor are potentially worse than overtreating a benign one, protocols for tumor removal or destruction and follow-up appropriate for a tumor of unknown biologic potential will need to be developed. Third, the indeterminate category is, in effect, a confession that histopathologic classification is not absolute and that improved methods of predicting biologic behavior of tumors are needed. While most pathologists appreciate the limitations of histopathologic interpretation, many clinicians have the mistaken belief that the process is absolute. An indeterminate category of melanocytic lesions should help improve communication with clinicians because it conveys the lack of certainty in diagnosis more clearly than terms such as atypical nevus or borderline melanoma.