Use of Digital Epiluminescence Microscopy to Help Define the Edge of Lentigo Maligna

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Objective: To compare identification of the border of lentigo maligna (LM) with digital epiluminescence microscopy (DELM) with clinical and Wood light assessment.

Results: The border determined by clinical examination was smaller than that determined with the Wood lamp or by DELM. Most lesions underwent an additional excision 5 mm beyond the DELM-defined border. The DELM pattern of LM with asymmetric follicular openings and dark brown rhomboidal structures changed at the periphery and became a pigmented thin mesh that was associated with the histopathological features of melanoma in situ. More homogeneous pigmented areas extending from the LM were associated with the pathologic features of melanocytic hyperplasia.

Conclusions: Visualization of LM by DELM (dermoscopy) helps to guide resection. Because LM arises in sun-damaged skin with melanocytic hyperplasia, determining the tumor-free margin requires the judgment of an experienced physician.

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Determining the Border of LM of the Head and Neck

Clinical Findings

<table>
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<tr>
<th>Border†</th>
<th>vs Wood Lamp</th>
<th>vs DELM</th>
<th>Wood Lamp vs DELM Findings</th>
<th>DELM vs Wood Lamp Findings</th>
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</thead>
<tbody>
<tr>
<td>Same</td>
<td>0/26</td>
<td>0/26</td>
<td>5/26</td>
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<tr>
<td>Greater</td>
<td>0/26</td>
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<td>2/26</td>
<td>19/26‡</td>
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<tr>
<td>Less</td>
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Abbreviations: DELM, digital epiluminescence microscopy; LM, lentigo maligna.
*N = 26. Data are expressed as numbers of lesions.
†This measurement process is described in the “Analysis” subsection of the “Methods” section.
‡Indicates differences were statistically significant (P < .001).

DIGITAL EPILUMINESCENCE MICROSCOPY

The DELM system offers a maximum field of view of 1 cm with 30-fold magnification. When the diameter of the melanocytic skin lesions in this study exceeded the field of view of the electronic camera, the clinical borders were segmented by the clock face and marked on the skin surface with gentian violet. The DELM images were stored without compression in bitmap format. The pixel resolution of each image was 640 × 480 at 24-bit color depth. The 2 criteria specific for LM include asymmetric pigmented follicular openings and dark brown or black rhomboidal structures.5,6

SURGICAL PROCEDURE

The initial biopsy for histopathologic correlation of the DELM findings was made 2 mm beyond the margin defined by DELM. The vertical incision extended into the adipose tissue and below the depth of the hair follicles. The center of the specimen was prepared with fixed tissue examination to ensure that the center of the specimen did not contain invasive melanoma. The circumferential margin was excised in 1-cm segments with a width of 2 mm. These segments conformed to the images stored by DELM. The edges of the specimens were inked and oriented on a map. The specimens from the margins were processed with frozen sections with hematoxylin-eosin (H&E) staining. Additional immunohistologic staining with MART-1 (melanoma antigen recognized by T cells) was used when differentiation was not possible.7,8 If the H&E findings for the entire section were positive or negative, then MART-1 staining was not performed. When there were equivocal areas, the MART-1 staining was performed. If there were areas that remained equivocal after H&E and MART-1 staining, then another layer was obtained and the staining was repeated. An excisional strip, which was about 1.0 × 0.3 cm in diameter, was taken from the ipsilateral or the contralateral preauricular cheek skin. The slides from the margins were processed with hematoxylin-eosin (H&E) staining, then another layer was obtained and the staining was repeated. An excisional strip, which was about 1.0 × 0.3 cm in diameter, was taken from the ipsilateral or the contralateral sun-exposed preauricular cheek skin and was used for comparison as a negative control specimen.

If a surgical margin was positive, the area was excised with subsequent stages of 3-mm margins and the specimens were similarly processed. At the conclusion of the procedure, the border of the excised area was traced onto a clear plastic sheet.

HISTOPATHOLOGIC CRITERIA

The criteria used for the diagnosis of a positive or negative margin were those established by Weyers et al for the diagnosis of melanoma in situ. The presence of melanocytic nests, nonuniform distribution of melanocytes, melanocytes far down the adnexal structures, melanocytes above the dermoepidermal junction, and melanocytic atypia defined melanoma in situ.9 Furthermore, the nests contained at least 3 atypical melanocytes. The nonuniform pigmentation of cells along the basement membrane was interpreted as crowding. Atypical melanocytes are those with mitoses, pleomorphic nuclei, or pleomorphic shape. There was epidermal atrophy and effacement of the rete ridges.10 Melanocytic hyperplasia with isolated atypical melanocytes was a common finding in sun-damaged skin found in the biopsy specimen of the contralateral preauricular cheek skin. The slides from the excised margin were reviewed in the context of the negative control from the sun-damaged skin of the contralateral cheek.

ANALYSIS

Initially, the 4 sheets from a single case were overlaid to observe whether the outlined area of the top sheet was the same, larger, or smaller than that of the bottom sheet. The observer was masked to the identity of the 2 clear plastic sheets. The 4 clear plastic sheets from each case were scanned, and image analysis of the digital photomicrography (NIH Image Analysis, vers. 1.62; National Institutes of Health, Bethesda, Md) was used to determine the surface area in square centimeters.

The DELM images of the 26 cases were reviewed for image quality and duplication. In randomly selected cases, the DELM image was compared with the H&E- and MART-1–stained frozen sections of the margins.

RESULTS

CHARACTERIZATION OF THE POPULATION

The mean age of the study population was 62 years (range, 43-74 years). Seventeen subjects were men and 9 were women. The most common lesion location was the cheek (7 patients), followed by the scalp (5), nose (4), forehead/ temple (4), ears (3), neck (2), and eyelid (1).

BORDERS OF LM AND MEAN SURFACE AREA

The border determined by results of visual clinical examination was smaller than that determined by use of the Wood lamp or by DELM findings (P < .001) (Table). In cases, the border identified by the Wood lamp and DELM findings was the same. The border determined by DELM was significantly greater than the one defined by the Wood lamp (P < .001). Comparison between groups is based on the χ² test; in all cases a 2-sided α level of less than .05 is considered statistically significant.

The 2 greatest diameters of the LM identified by clinical assessment had a mean of 2.4 × 2.2 cm; by the Wood lamp, 2.8 × 2.4 cm; and by DELM, 3.0 × 2.7 cm. The initial excision for pathological correlation of the edge of the process was marked on the skin before incision (3.2 × 2.9 cm).
cm). Fifteen lesions required a second stage of surgery with an additional 3-mm margin, and 9 needed 3 stages of surgery. Thus, most of the lesions had approximately 5-mm margins removed beyond the margins determined by DELM. Some inaccuracy occurred in calculating the area excised because the edges of the defect retracted after excision; however, the 2 mean diameters of resection were 3.5 × 3.3 cm. Although this discrepancy should be uniform across all lesions, some regional differences may be found, depending on tissue laxity and position. All measurements were performed in a supine position. The mean (SD) surface area identified by clinical assessment (5.28 [1.9] cm²) was less than that determined with the use of the Wood lamp (6.72 [1.5] cm²), which was significantly less than the area identified by DELM (8.1 [2.1] cm²) \((P =.01)\). There was a significant difference between the area identified by DELM and the resected area (11.6 [2.7] cm²) \((P =.01)\).

### DELM AND HISTOLOGIC COMPARISON

Six cases were randomly selected for comparison of the histologic findings with the DELM images. Thirty-five histologic slides prepared with H&E staining from these 6 cases were reviewed. Fourteen of these specimens also underwent immunohistologic staining with MART-1. The DELM pigment pattern of the center of the LM changed at the periphery, becoming a pigmented thin mesh (Figure 1A). The central area of LM demonstrating pigmented follicular openings and rhomboidal structures was associated with the pathologic features of melanoma in situ (Figure 2A and Figure 3A). The peripheral areas of the LM showed scattered nests at the dermoepidermal junction with at least 3 atypical melanocytes and crowding of the non-
uniform pigment cells along the basement membrane (Figure 2B). When the LM blended into uniform hyper-pigmented areas of solar changes at the periphery of the lesion, melanocytic hyperplasia was present (Figures 2B, 3B, 4, and 5C). None of the cases had invasive melanoma in the center of the specimen.

**COMMENT**

Digital epiluminescence microscopy, which is used primarily for diagnosis of pigmented lesions, may help to improve the clinical identification of melanoma in situ. The mean surface area excised for the LM cases in this study was greater than the DELM-determined area. During the past decade, there has been a debate over the use of frozen sections to interpret the resection margins of melanoma. The clinicopathologic correlations reported in this study may help to resolve concerns. Consistency of the frozen sections requires preparation of 2- to 4-µm-thick sections without artifacts or distortion. Thick sections provide multilayered specimens that make cell borders and cytologic characteristics of individual cells indistinct. Immunostaining has become a useful adjunct to H&E preparation in increasing sensitivity and specificity of melanoma on frozen sections.11-13

Sun-exposed areas may have an increased number of melanocytes in the basal layer of the epidermis, and some of these melanocytes may be cytologically atypical.14 The diagnosis of a positive margin is based on increased numbers of atypical melanocytes within the basal layer and melanocytes disposed as single units above the basal layer. Interpretation of the histopathologic material as to whether the increased number of atypical melanocytes indicates the presence of LM or solar-induced melanocytic hyperplasia may be enhanced by comparison with a biopsy specimen from the contralateral area of sun-exposed skin.15 There is variability in interobserver concordance by dermatopathologists with re-

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**Figure 3.** A, The digital epiluminescence microscopy (DELM) area with brown globules to the left of the arrow in Figure 4B has multiple nests of atypical melanocytes at the dermoepidermal junction, consistent with lentigo maligna. (MART-1 [melanoma antigen recognized by T cells], original magnification ×40). B, The area to the right of the arrow in Figure 4B has scattered nests of melanocytes at the dermoepidermal junction and crowding of the nonuniform pigment cells along the basement membrane, consistent with melanocytic hyperplasia (frozen sections, MART-1, original magnification ×20).

**Figure 4.** Digital epiluminescence microscopy (DELM) encompassed a lentigo maligna (LM) of the left cheek, which arises within sun-damaged skin. The LM has a central scar from a previous punch biopsy and shows brown globules and dots within the lesion. Although most of the LM border is readily demarcated by its pigmentary pattern, the superior portion blends into a less distinct area of pigmentation (small arrow on the clinical photograph [A] and large arrow on the DELM image [B]).
pect to interpretation of the histopathologic specimens prepared with H&E staining of pigmented lesions.15-21

I have used the histopathologic criteria for more than 10 years.7 Support for the clinicopathologic findings at the edges of LM reported herein can be found during the examination of excision margins of melanomas with H&E staining in paraffin-embedded sections. Examination of 10 LM melanomas by Breuninger et al22 found a clearly demonstrable, uninterrupted spread of groups of atypical melanocytes into the periphery at the dermoepidermal junction. In LM melanomas, there was a 54% probability of finding these groups of atypical cells 5 mm beyond the clinical border, and the median safety margin was 8 mm.22 This 5- to 8-mm margin obtained from paraffin-embedded sections is greater than the border identified by DELM and similar to the margin of resection in this study.

An additional consideration is the accuracy of the histopathologic correlation with DELM. In this study, correlation of the histologic findings with DELM interpretation was performed retrospectively; therefore, the DELM findings did not influence the interpretation of the histopathologic findings. Many researchers have examined the histopathologic correlation of the dermoscopic structures.23-31 A variety of orientation methods, including orienting sutures and micropunch, were used. It does not seem that DELM was previously used to mark the excisional units. Combining DELM with the standard tissue-orienting approach of Mohs surgery provided an opportunity to correlate clinical findings with DELM images and histopathologic findings. In this study, the histopathologic identification of melanoma in situ was aided by immunohistochemical staining of sections with MART-1, a marker for melanocytic differentiation.7,8,11-13,32-34 The increased sensitivity of MART-1 helped enhance the interpretation of specimens at the edge of the area defined by DELM.8,33

In the hands of an experienced physician, dermoscopy has been shown to increase diagnostic accuracy com-

Figure 5. A, Three sites on the edge of a lentigo maligna (LM) of the cheek are shown on digital epiluminescence microscopy (DELM) images. B, Sharp definition of the edge. C, The LM border blends into an area of homogeneous pigmentation. D, The lateral island of pigmentation is part of the LM.
pared with visual clinical inspection.\textsuperscript{35-41} Given the increased diagnostic accuracy afforded with dermoscopy of pigmented lesions, it is not surprising that DLEM enhances the diagnostic accuracy of determining the clinical margin of a lesion. Most criteria for epiluminescence-microscopy diagnosis of pigmented lesions are network-derived features such as a broad or a thickened network, which depend on rete ridge pattern at the dermoeidermal junction. The atrophic epidermis of the sun-exposed face has flattened rete ridges; hence, the pigment pattern at the edge of the LM fades into the atrophic epidermis. The ease of use of dermoscopy makes the technique described in this report readily available to clinicians, who use visual inspection to guide the margin of resection. Although confocal microscopy, an emerging technology, also offers promise in defining tumor margins, it is currently less widely available than dermoscopy.\textsuperscript{42}

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Dr Robinson was not involved in the editorial evaluation or editorial decision to accept this work for publication. Correspondence: June K. Robinson, MD, Division of Dermatology, Cardinal Bernardin Cancer Center, Loyola University Stritch School of Medicine, 2160 S First Ave, Room 341, Maywood, IL 60153 (jrobin5@lumc.edu).

REFERENCES