Improving Management and Patient Care in Lentigo Maligna by Mapping With In Vivo Confocal Microscopy

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**Importance:** Lentigo maligna (LM) is a clinical, pathologic, and therapeutic challenge with a higher risk of local recurrence than other types of melanoma correctly treated and also carries the cosmetically sensitive localization of head and neck.

**Objective:** To determine whether in vivo reflectance confocal microscopy (RCM) mapping of difficult LM cases might alter patient care and management.

**Design:** Analysis of LM and LM melanoma (LMM) in a series of patients with large facial lesions requiring complex reconstructive surgery and/or recurrent or poorly delineated lesions at any body sites were investigated.

**Settings:** Two tertiary referral melanoma centers in Sydney, Australia.

**Participants:** Thirty-seven patients with LM (including 5 with LMM) were mapped with RCM. Fifteen patients had a recurrent LM, including 9 with multiple prior recurrences. The LM was classified amelanotic in 10 patients, lightly pigmented in 9, and partially pigmented in 18.

**Interventions:** The RCM images were obtained in 4 radial directions (allowing for anatomic barriers) for LM margin delineation using an RCM LM score previously described by our research team.

**Main Outcome Measures:** Differences in the margin of LM as determined by RCM vs dermoscopy vs histopathologic analysis.

**Results:** Seventeen of 29 patients (59%) with dermoscopically visible lesions had subclinical (RCM-identified) disease evident more than 5 mm beyond the dermoscopy margin (ie, beyond the excision margin recommended in published guidelines). The RCM mapping changed the management in 27 patients (73%): 11 patients had a major change in their surgical procedure, and 16 were offered radiotherapy or imiquimod treatment as a consequence of the RCM findings. Treatment was surgical in 17 of 37 patients. Surgical excision margins (based on the RCM mapping) were histopathologically involved in only 2 patients, each of whom had an LM lesion larger than 6 cm.

**Conclusions and Relevance:** In vivo RCM can provide valuable information facilitating optimal patient care management.


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to differentiate LM from other pigmentation of the face,\textsuperscript{5,6} and can assist in defining peripheral margins of LM even in amelanotic tumors.\textsuperscript{7,8} Recently, RCM features that can distinguish LM from benign macules were described in a large series of 347 facial pigmented lesions. Using this data set, we previously developed and validated an algorithm (LM score) to distinguish LM from BM.\textsuperscript{9} In the present study, we performed a retrospective review of patients referred to 2 melanoma treatment centers with LM that presented management challenges. The cases were classified as difficult because the lesions were lightly colored or amelanotic or because they had recurred after prior treatment. The aims of the study were to describe the use of RCM in the clinical management of this challenging group of LM cases and to determine if the RCM findings affected management and outcomes.

**METHODS**

**PATIENTS**

Challenging cases of LM and LMM were referred for in vivo RCM at the Sydney Melanoma Diagnostic Centre and Melanoma Institute Australia (in vivo RCM performed by P.G.). Patients were referred because of the presence of 1 or more of the following: facial involvement of an LM lesion larger than 5 cm that would require complex reconstructive surgery; recurrent LM; or lightly pigmented or poorly delineated LM. Between 2006 and 2010, a total of 37 consecutive patients (26 women and 11 men), aged 47 to 88 years (mean age, 71 years) were assessed by RCM for mapping of the area to be treated. All patients were assessed by a multidisciplinary team (usually at a specialized multidisciplinary lentigo maligna clinic) including at least 1 dermatologist (P.G., F.J.M., or S.W.M.), 1 plastic surgeon (J.R.S. or M.J.Q.), and 1 radiation oncologist (A.H. or G.F.). Thirty-two patients had been diagnosed as having LM, and 5 as having LMM. Fifteen patients with LM presented with recurrent LM, including 9 who had already had multiple recurrences. Twenty-two patients were referred for evaluation and treatment following an initial diagnosis of LM.

Regarding their clinical appearance, 10 LM lesions were amelanotic (including 9 lesions invisible to naked-eye or dermoscopic assessment and 1 pink lesion); 9 were partially lightly pigmented; and 18 were pigmented or partially pigmented. These color categories were defined according to the definitions as previously described by Menzies et al.\textsuperscript{10} Twenty-seven LM lesions were on the cheek, 5 on the nose, 2 on the temple, 1 on the eyebrow, 1 on the shoulder, and 1 on the lower leg.

**INSTRUMENTS AND ACQUISITION PROCEDURE**

A baseline clinical photograph was taken, and clinical margins were determined with the aid of dermoscopy. In vivo RCM images were acquired by means of a near-infrared reflectance confocal laser scanning microscope (Vivascope 1500; Lucid Inc) that uses an 830-nm laser beam with a maximum power of 35 mW. Instrument and acquisition procedures are described elsewhere.\textsuperscript{9,11} Each image corresponds to a horizontal section at a preselected depth with a 0.5 \( \times \) 0.5-mm field of view, a lateral resolution of 1.0 \( \mu \)m, and axial resolution of 3 to 5 \( \mu \)m. A sequence of montage images (“mosaic” images) were acquired for each lesion at the level of the dermoepidermal junction to explore a minimum field of view of 4 \( \times \) 4 mm and a maximum of 8 \( \times \) 8 mm per lesion. The device was centered on the lesion for larger lesions that were not completely encompassed within the field of view and then moved to several locations if the lesion was not homogenous. Confocal images were obtained in 4 radial directions (allowing for anatomical barriers) for margin determination until no evidence of LM was seen. At least 1 mosaic of RCM images was obtained from the center of each lesion, and then additional mosaic or sites were obtained in 4 radial directions until the mosaic showed no features of lentigo maligna in each of the 4 directions. Two- or 3-mm punch biopsies were often performed, particularly at equivocal sites. In this way, margin determination may have occurred over serial consultations. The margins were explored in only 4 directions because of the time necessary to capture each montage. The median and average number of sites (mosaics) examined was 6 per patient. The extent of the lesion was marked on the skin of the patient with a surgical pen and photographed at the time of the mapping (Figure 1). This clinical photograph was reviewed by surgeons, radiation oncologists, and any other clinicians involved in the patient’s care.

**Histopathologic Analysis**

The diagnosis of LM was always established by histopathologic analysis. Each of the 37 patients had at least 1 positive site and 1 negative site biopsied to obtain histopathologic correlation (total number of biopsies per patient ranged from 2 to 12; median, 5; mean, 5). Targeted 2- or 3-mm punch biopsies were often performed at the margins of the lesion, in particular when they were considered equivocal by RCM (eg, sparse atypical cells at the dermoepidermal junction). Pathologic as-
The lesion were on average 60% smaller than the final margins. Reflectance confocal microscopy (RCM) assessed as being greater or less than 5 mm. Melanoma management guidelines in the United States, Australia and New Zealand, determined in millimeters. However, the images were evaluated by the treating clinicians, and the differences were assessed as being greater or less than 5 mm. Melanoma management guidelines in the United States, Australia and New Zealand, and in some areas of Europe recommend a 5-mm excision margin for LM. This study was approved by the human ethics review committee (Sydney South West Area Health Service Ethics Review Committee protocol No. X03-0218). All clinical investigations were conducted according to the Declaration of Helsinki principles.

**RESULTS**

**RCM AND HISTOPATHOLOGIC CORRELATION**

The histopathologic findings and diagnostic correlation with the RCM features in nearly all cases. In total, 185 punch biopsies were performed during the mapping of the 37 patients (median, 5 biopsies per patient). There were 4 false-positive sites (diagnosed as an LM area by the LM score on RCM and not confirmed by pathologic findings) and 5 false-negative sites (diagnosed as LM by histopathologic study but not by the RCM assessment). Seventeen of 29 patients with visible lesions (1 pink, 9 light-colored, and 18 partially or totally pigmented) had evidence of subclinical disease more than 5 mm beyond the edge of the clinical and/or dermoscopically identified margin. The results are summarized in the Table.

Both length and width of the clinically visible area of the lesion were on average 60% smaller than the final corresponding dimensions determined by RCM assessment. Thus, the visible area was on average less than 40% of the area that was treated based on the RCM mapping findings.

**MANAGEMENT**

Complete surgical excision of the lesion was recommended on 3 occasions because there were RCM features raising the possibility of focal dermal invasive melanoma. No invasive melanoma was identified on subsequent histopathologic evaluation of these 3 cases.

Management was influenced by the RCM mapping in 27 of 37 patients. Eleven patients had a change in their surgical management (a different reconstruction was undertaken because the area to be treated was more extensive than the one delineated by the 5-mm margins around the naked-eye and/or dermoscopically visible area). In 13 patients, the LM was treated with radiotherapy, which had been recommended prior to RCM mapping in 4 patients. Importantly, 9 were selected for this mode of treatment by the multidisciplinary management team because surgical excision and reconstruction were considered too challenging cosmetically only after RCM mapping. Seven patients opted for medical treatment (imiquimod, 5%, applied 5 times a week for 3 months), having declined surgery and radiotherapy.

**OUTCOMES**

Outcomes of the mapping intervention were determined with a median follow-up of 37 months (range, 7-66 months). Treatment was surgical in 17 of 37 patients, and RCM-delineated margins were involved after excision in 2 patients. These 2 patients had large (>6 cm) LM lesions on their cheeks (Figures 2, 3, 4, and 5). Mapping

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<th>Table: Pathologic, RCM, and Dermoscopic Correlations</th>
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<tbody>
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<tr>
<td>Pathologic Analysis</td>
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<td>Dermoscopic Evaluation</td>
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<td>RCM LM Score</td>
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**Abbreviations:** FP, false-positive site; FN, false-negative site; LM, lentigo maligna; RCM, reflectance confocal microscopy. $^A$All FN sites by RCM were also FN by dermoscopy. $^B$None of the 4 FP sites by RCM were also FP by dermoscopy. For all cases of discordance between the RCM and pathologic analysis, the features of both the RCM and pathologic evaluations were reconsidered. In each case there were scattered atypical melanocytes, and it appeared that the differences in interpretation occurred because of sampling issues or differences in the minimal threshold applied for a diagnosis of LM by the physicians using the RCM and pathologic analysis techniques.
Figure 3. Images corresponding to area X in Figure 2. A, Dermoscopy image of the area of biopsy-proven lentigo maligna (LM). There are some areas of slate-gray globules and rhomboidal structures, and there are some asymmetric pigmented follicular structures (upper arrow). The lower arrow indicates artifact from a previous punch biopsy. B, Reflectance confocal microscopy (RCM) 8 × 8-mm mosaic at the dermoeidermal junction showing some atypical melanocytic cells and the distortion of the dermoeidermal junction (inset, detail of an RCM image, 0.5 × 0.5 mm, showing numerous bright, large [~20 µm], irregularly shaped cells [arrows] with a complete disorganization of the junction [noded papillae]). C, Hematoxylin-eosin–stained specimen from the RCM field showing a lentiginous proliferation of atypical melanocytes with involvement of a skin appendage in chronically sun-damaged skin (original magnification ×100). These features are characteristic of LM.

Figure 4. Images corresponding to area Y in Figure 2. A, Dermoscopy image showing ill-defined pigmentation with no clear feature of lentigo maligna (LM). B, Confocal image, 0.5 × 0.5 mm, showing a continuous layer of small monomorphous cells that are not very bright at the spinous layer. C, Hematoxylin-eosin–stained specimen from the RCM field showing atrophic epidermis with loss of rete ridges and severe dermal solar elastosis; there is mild patchy basal keratinocyte pigmentation but no evidence of LM (original magnification ×100).
in only 4 radial directions is helpful but certainly less precise when the surface is large. None of the patients treated by surgery had developed recurrences by last follow-up.

Treatment was nonsurgical in 20 of 37 patients (13 patients received radiotherapy, while 7 were treated with imiquimod). Recurrence was suspected in 1 imiquimod-treated patient. In this patient, follow-up RCM showed scattered atypical cells. A random biopsy was performed at 1-year follow-up because this LM was consistently amelanotic and had already recurred 5 times previously (treated by surgery on each occasion). The biopsy showed scattered mildly atypical melanocytes suggestive of early LM. The patient was again treated with imiquimod, and no recurrence was detected with RCM after 40 months of follow-up.

One patient who did not have complete clearance of the lesion 12 months after initial treatment with radiotherapy had the lesion surgically excised (with histopathologically confirmed residual LM) and had no recurrence 40 months later. Two patients treated by radiotherapy developed recurrences 24 and 36 months after treatment, respectively. The recurrences in both of these patients were very near to or on the mucosal eyelid where the RCM mapping and the radiotherapy field were limited. The recurrences were detected with RCM and were treated with imiquimod in one case and surgery in the other.

**COMMENT**

Determination of the peripheral margins of LM is difficult from both the clinical and pathologic perspectives. Clinically, LM is often amelanotic peripherally and can spread far beyond the visible margins. Pathologically, there is some heterogeneity in the histopathologic features of LM in different parts of the lesion. Moreover, at the margins, the significance of scattered mildly atypical cells may not be appreciated, and they can be difficult to differentiate from melanocytic hyperplasia in sun-damaged skin. There are no prospective studies or randomized controlled trials available to form the basis for any recommendations for the management of LM. Mohs surgery and staged excision have been proposed as techniques to delineate the margins of LM more precisely, but these procedures are expensive, require a high degree of expertise, and are not universally accepted. In vivo RCM appears to be a streamlined and less invasive alternative to these methods. Topical treatment and radiotherapy have also been proposed to treat larger fields, with the hope of decreasing recurrence rates and having less cosmetic sequelae.

Recurrences of LM are often at the margins of the previously treated area. There are 3 lines of evidence to support this observation. Agarwal-Antal et al suggest that 5-mm margins would clear less than 50% of the LM lesion. When they performed staged excision in 92 cases with 5-mm margins, 42% of tumors were clear after the first stage, and 69% after the second. In a large radiotherapy retrospective study, Farshad et al found 5 recurrences in 96 patients treated; 4 were at the edge of the margins field that was 1 cm larger than the clinical
lesion. Finally, Cotter et al used an imiquimod protocol to treat LM with 2-cm margins. Nevertheless, 90% of the recurrences occurred at the edge of the margins field that was 2-cm larger than the visible lesion.

Clinical follow-up of previously treated LM is often unreliable because of the development of nonspecific pigmentation arising from treatment-induced inflammation. In particular, pigment incontinence (ie, pigment-laden macrophages in the superficial dermis that phagocytose melanin originating from damaged basal epithelial cells) is a well-known pitfall associated with clinically detecting a suspected recurrence. In contrast, amelanotic recurrences have also been well documented.

In view of this evidence, we hypothesized that RCM might be a useful tool not only to map the area for treatment but also to detect disease recurrence. Dermoscopy and Wood light examination have been described as useful techniques to better define the extent and recurrence of LM. However, in vivo RCM appears to be superior because it provides cellular resolution in the upper layers of the skin. Because melanin and/or melanosomes appear “bright” under reflectance at near-infrared wavelengths, pigmented cells are easily visualized, and RCM features can be evaluated for diagnosis. Importantly, RCM generates a horizontal view up to 8 × 8 mm and it is therefore possible to assess more of the lesion using this technique than with pathological assessment of vertically oriented small biopsy specimens (even with step sectioning) that are usually examined in routine histopathologic analysis. On the contrary, determining in horizontal sections the exact site of the cells (junctional or dermal) is not easy, especially when the dermoepidermal junction is very distorted by atypical cells. In this series, on 3 occasions RCM features raised the possibility of focal dermal invasive melanoma, but it was not identified on subsequent histopathologic evaluation. The reason for the apparent discrepancy between the RCM and pathologic findings may be also related to sampling such that the dermal component identified on RCM was not present in the pathology biopsy sections.

The series described herein demonstrates that RCM can aid in mapping difficult (including recurrent) LM lesions, even when they are lightly colored or amelanotic. A further benefit of this in vivo technique is that it can be used to assess the density and degree of cytological atypia of the proliferation of melanocytic cells. As is well recognized from histopathologic studies, there appears to be a continuum from melanocytic hyperplasia of sun-damaged skin to the presence of a mildly atypical hyperplasia to a lentiginous junctional proliferation of atypical cells to fully transformed melanoma in situ with nests and florid pagetoid spread. Further studies are necessary to investigate whether RCM might be used to grade the degree of atypia and extent of melanocytic proliferation and to correlate this with the histopathologic features to provide a more reliable classification system and potentially a basis for offering different treatment choices. It is important to emphasize that histopathologic analysis is not without its difficulties in diagnosing early LM, distinguishing it from melanocytic proliferations in acutely damaged skin, and in assessing LM margins, and these same difficulties are also encountered with RCM.

To determine whether recently proposed thresholds of melanocyte density for diagnosing LM by histopathologic analysis are also applicable to RCM diagnosis will require further study.

The major limitation of RCM is the significant time and expertise necessary to map a large area: the assessment of an area of 8 × 8 mm by an RCM expert would require at least 5 minutes. Thus, assessing half a cheek can take 1 hour. Another issue is the difficulties associated with imaging some anatomic locations. Use of the Vivascope 1500 requires a 2-cm adhesive ring to be pasted on the area to be examined. The head of the microscope must be maintained in this ring for the entire time required to record all the images necessary for diagnostic evaluation. The new, light, handheld confocal microscopes (eg, VivaScope 3000, Lucid Inc) do not require the use of an adhesive ring. They can move freely, saving time in the process and resolving the limitation of usage around the nostril and eye or behind the ear. The last issue is the price of the technology and expertise required because the costs of RCM are not yet reimbursed. In our opinion, RCM is probably of most value in LM management because it gives the clinician all the information required to optimally plan treatment (surgical, radiation, or topical).

In conclusion, RCM is a noninvasive imaging technique likely to enhance the accuracy of clinical diagnosis of LM and delineate its margins. As demonstrated by the results of this study, it may also help to guide overall case management. Furthermore, the information obtained by this technique can assist multidisciplinary medical teams in their management of difficult and challenging cases. Nevertheless, the results of randomized prospective studies will ultimately be required to provide the best level of evidence for making management recommendations for patients with LM.

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REFERENCES


18. Weyers W, Bosczkowiz M, Weyers I, Bittinger A, Schill WB. Melanoma in situ versus melanocytic hyperplasia in sun-damaged skin: assessment of the signifi-


