JAMA Dermatology | Original Investigation

Correlation of Handheld Reflectance Confocal Microscopy With Radial Video Mosaicing for Margin Mapping of Lentigo Maligna and Lentigo Maligna Melanoma

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IMPORTANCE The management of lentigo maligna (LM) and LM melanoma (LMM) is challenging because of extensive subclinical spread and its occurrence on cosmetically sensitive areas. Reflectance confocal microscopy (RCM) improves diagnostic accuracy for LM and LMM and can be used to delineate their margins.

OBJECTIVES To evaluate whether handheld RCM with radial video mosaicing (HRCM-RV) offers accurate presurgical assessment of LM and LMM margins.

DESIGN, SETTING, AND PARTICIPANTS This prospective study included consecutive patients with biopsy-proven LM and LMM located on the head and neck area who sought consultation for surgical management from March 1, 2016, through March 31, 2017, at the Dermatology Service of the Memorial Sloan Kettering Cancer Center. Thirty-two patients underwent imaging using HRCM-RV, and 22 patients with 23 LM or LMM lesions underwent staged surgery and contributed to the analysis.

MAIN OUTCOMES AND MEASURES Clinical lesion size and area, LM and LMM area based on HRCM-RV findings, surgical defect area estimated by HRCM-RV, and observed surgical defect area. In addition, the margins measured in millimeters estimated for tumor clearance in each quadrant based on HRCM-RV findings were calculated and compared with the surgical margins.

RESULTS Among the 22 patients (12 men and 10 women; mean [SD] age, 69.0 [8.6] years [range, 46-83 years]) with 23 lesions included in the final analysis, the mean (SD) surgical defect area estimated with HRCM-RV was 6.34 (4.02) cm^2 and the mean (SD) area of surgical excision with clear margins was 7.74 (5.28) cm^2. Overall, controlling for patient age and previous surgery, surgical margins were a mean of 0.76 mm (95% CI, 0.67-0.84 mm; $P < .001$) larger than the HRCM-RV estimate.

CONCLUSIONS AND RELEVANCE Mapping of LM and LMM with HRCM-RV estimated defects that were similar to but slightly smaller than those found in staged excision. Thus, mapping of LM using HRCM-RV can help spare healthy tissue by reducing the number of biopsies needed in clinically uncertain areas and may be used to plan treatment of LM and LMM and counsel patients appropriately.

Video

Supplemental content

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JAMA Dermatol. 2017;153(12):1278-1284. doi:10.1001/jamadermatol.2017.3114
Published online October 11, 2017.

Delineating the borders of lentigo maligna (LM) and LM melanoma (LMM) is challenging because they occur in heavily sun-damaged areas and may be hypomelanotic or amelanotic, with subclinical spread not visible to the naked eye.[1] This situation can lead to underestimation of surgical margins, resulting in positive surgical margins and subsequent recurrences.[2,3] In addition, because LM and LMM occur on cosmetically sensitive areas, such as the face, and can have extensive subclinical spread, complete margin assessment and control with techniques such as Mohs micrographic surgery, staged excision, or the spaghetti technique are important. Surgical margins needed to clear LM and LMM on the head and neck area are also greater than those needed for traditional trunk and extremity melanomas.[4-6]

Dermoscopy and Wood lamp examination have improved the delineation of LM and LMM margins.[7] However, false-positive and false-negative findings occur,[8] and scouting biopsies are often performed.[9,10] Scouting biopsies provide static information that can be difficult to contextualize because certain features, such as melanocytic hyperplasia, occur in benign, sun-exposed skin and at the trailing edge of LM and LMM. Reflectance confocal microscopy (RCM) is a noninvasive imaging system that allows in vivo cellular evaluation of the epidermis and upper dermis.[11,12] Reflectance confocal microscopy has a sensitivity of 85% and specificity of 76% to diagnose LM and LMM[13] and has been used to delineate their margins.[14,15] Most of this work has been based on traditional widefield-probe RCM, which requires attaching a metal ring on the skin and allows imaging of areas to 8 × 8 mm. However, LM and LMM can be larger than 8 mm and can occur at uneven surfaces where the metal ring may detach. Thus, mapping the LM and LMM margins with widefield-probe RCM can be a labor-intensive and time-consuming process (approximately 1 hour per lesion).[14] Conversely, handheld RCM (HRCM) can acquire images and videos along ad hoc, arbitrarily freeform, nonrastered paths and over large areas that the user determines in real time, allowing a live approach to mapping margins over large areas. Although HRCM allows less restrictive imaging acquisition, the data regarding its use in diagnosing and mapping LM and LMM are limited[15,16] owing mainly to the lack of orientation during image acquisition and lack of mosaicing capabilities in the HRCM software. However, with the advent of HRCM video mosaicing, dynamic videos can now be transformed into static video mosaics to help obtain architectural information.[17-19]

Herein, we describe a novel imaging technique for obtaining radial HRCM videos and video mosaics from the LM or LMM center to the periphery, guided by the use of adhesive paper rings. The aim of this study was to evaluate whether radial imaging offers accurate presurgical assessment for LM and LMM margins and anticipates surgical defects.

Key Points

Question Is handheld reflectance confocal microscopy with radial video mosaicing accurate for noninvasive delineatation of subclinical lentigo maligna and lentigo maligna melanoma borders and estimation of its margins?

Findings In this study, 23 lentigo maligna and lentigo maligna melanoma lesions from 22 patients were evaluated. Overall, controlling for patient age and previous surgery, surgical margins were a mean of 0.76 mm larger than the estimate obtained with reflectance confocal microscopy.

Meaning Handheld reflectance confocal microscopy with radial video mosaicing findings correlated well with histologic findings and may be useful in planning treatment and counseling patients.

Methods

Clinical Information

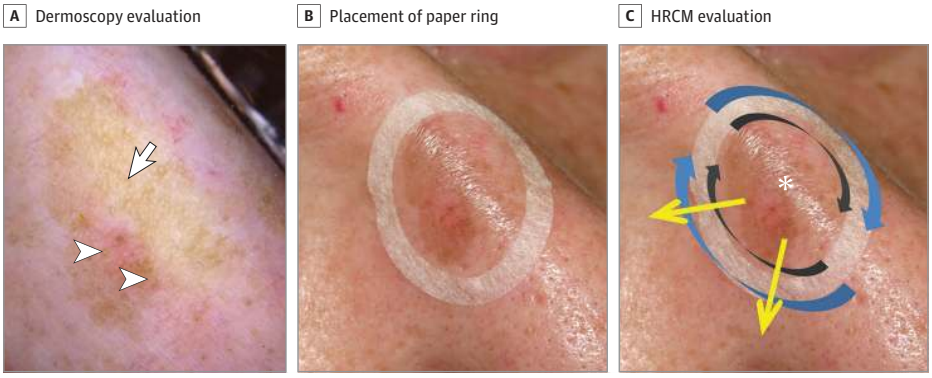
From March 1, 2016, through March 31, 2017, we conducted a prospective study including consecutive patients with LM and/or LMM who consulted for surgical advice in the Dermatology Service of Memorial Sloan Kettering Cancer Center, New York, New York. We included patients who had biopsy-proven LM or LMM located in the head and neck area and who underwent imaging using HRCM radial video mosaicing (HRCM-RV) before staged excision with complete circumferential margin control. We excluded patients with LM and LMM not located in the head and neck area, patients who decided not to undergo staged excision, and patients who pursued treatment elsewhere. This study was approved by the institutional review board of Memorial Sloan Kettering Cancer Center. All patients provided written informed consent to imaging with confocal microscopy and to have surgery performed.

We collected patient demographic information, prior LM and LMM treatments, lesion location, Breslow thickness, and numeric surgical stages. We calculated the clinical lesion size and area, the LM or LMM area based on HRCM-RV findings, the surgical defect area estimated using HRCM-RV, and the observed surgical defect area. To calculate the areas, we obtained digital images of the lesions and surgical defects using a digital camera (PowerShot G10; Canon, Inc) or a dermoscopy system (Veos DS3; Canfield Scientific). Using Mirror software (version 7.5; Canfield Scientific), we retrieved the images and calculated the areas after image calibration with a ruler placed in the image field, anthropometric measurements, or annotations in the medical record, such as defect size. To ensure measurement accuracy, 2 calibration methods were used for each image.

Clinical Margin Delineation and Confocal Imaging

Clinical margins were determined using dermoscopy and Wood lamp examination. To facilitate RCM navigation and margin calculation, we surrounded this margin with adhesive paper rings (product number 1529; 3M)[20] (**Figure 1**). In irregularly shaped lesions, multiple paper rings were placed and eventually overlapped to allocate the entire lesion inside the paper rings and image its entire border. Several paper ring diameters are available; however, all paper rings have a rim width of 2.5 mm, which was used to calculate the estimated surgical margins. We imaged the lesions with an HRCM device (VivaScope 3000; Caliber Imaging and Diagnostics), which has a lateral resolution of approximately 1 µm, optical sectioning of

Figure 1. Lentigo Maligna (LM) Margin Determination Using Dermoscopy, Wood Lamp Examination, and Handheld Reflectance Confocal Microscopy (HRCM)



A, Dermoscopy evaluation reveals a pigmented lesion with ill-defined margins, asymmetric pigmented follicular openings (arrow), and a circle within a circle (arrowheads). B, Paper ring placed outside the clinical margin determined with dermoscopy and Wood lamp examination to facilitate confocal navigation. C, The HRCM evaluation was performed by initially imaging the center to determine the cellular morphologic features (asterisk), and later by imaging clockwise the peripheral margin inside (black arrows) and outside (blue arrows) the ring. Radial videos were obtained (yellow arrows) in the areas where a higher degree of atypia was identified outside the paper ring to determine the LM or LM melanoma subclinical extension.

approximately 3 µm, and a field of view of 1 × 1 mm. Two of us (O.Y. and M.C.) with 1 year and more than 5 years of experience, respectively, performed image acquisition together for the first 10 cases, and later acquired images together or alone depending on availability in the clinic.

Initially, we imaged the lesion center to identify the predominant morphologic features of the melanoma cell (large round, dendritic, or pleomorphic).[13] Next, we navigated clockwise along the ring's inner margin to confirm the dermoscopic or Wood lamp margins. Then, we imaged along the outer ring margin to identify LM or LMM subclinical spread. When a positive area was identified outside the ring, we captured a video at the plane where the highest degree of atypia was identified and navigated radially from the lesion center toward the LM-positive area outside the ring until no LM features were observed (Figure 1C). Radial videos were obtained in all the quadrants that were positive outside the paper ring.

To consider an area as positive on HRCM, the features present in the LM algorithm (**Table**),[13,15] validated to diagnose LM using widefield-probe RCM and HRCM, were used.[13,16] When further evaluating the periphery, margins were considered to be positive for LM if any of the positive criteria of the LM algorithm were present and if a single large round or large dendritic cell was identified on RCM.[15] Based on previous studies and the results obtained when imaging complex LM and LMM cases,[17] we also considered the margin to be positive if smaller atypical dendritic cells were observed continuing from the LM trailing edge (Table).

Determination of Subclinical Tumor Margins Using HRCM-RV

To calculate subclinical margins with HRCM-RV, the acquired videos were converted into video mosaics using an algorithm written in MATLAB (MathWorks).[18] In brief, frames were extracted and then stitched together to create mosaics of the imaged area. Video mosaics were used to calculate the subsurface extension by measuring the distance between the inner part of the paper ring (clinical margin) and the furthest HRCM-positive finding identified (confocal margin) (**Figure 2** and **Video**). To determine these measurements, the following 2 reference variables were used: (1) the ring rim width of 2.5 mm and (2) each HRCM field of view of 1 × 1 mm, which equals 1000 × 1000 pixels. We overlaid these measurements on the clinical pictures and calculated the HRCM-estimated area. Margin determination was performed by one of us (O.Y.) with experience in RCM and dermatopathology by reviewing the radial video mosaics obtained in each quadrant.

Staged Excision and Determination of Surgical Margins Using HRCM-RV

According to our practice protocol and standard of care,[4,21,22] 3 of us (K.S.N., E.L., and A.M.R.) performed staged excisions blinded to HRCM calculations with an initial 5-mm margin beyond the clinical margin, which the surgeons determined using dermoscopy and Wood lamp examination. After radial histopathologic sectioning, further excision stages guided by histopathologic findings were performed until reaching at least 3 mm of histologic clearance.[4,21,22] Thus, before surgery and based on these variables, we determined the estimated surgical defects from the HRCM-RV findings. A hypothetical LM or LMM extending just to but not beyond the outer edge of the paper ring would yield 2.5 mm of histologic clearance rather than 3 mm. However, we considered the 0.5 mm to be negligible because such variations occur depending on the surgical technique.[23] If a quadrant was positive outside the ring on HRCM, we added 3 mm to the margin calculated using HRCM-RV (eFigure in the Supplement).

Statistical Analysis

Descriptive statistics, such as means, SDs, medians, ranges, and relative frequencies, were used to describe the patient, lesion, imaging, and surgical characteristics. Normality of

interval-scaled variables was assessed using graphic methods. The imaging data were assessed using the following 2 approaches: a lesion-based approach, with each lesion contributing 1 pair of surgical and HRCM measures (total lesion area in square centimeters), and a radial quadrant-based approach, with each lesion contributing 4 pairs of surgical and HRCM measures of length (in millimeters) based on the distances calculated emanating from the clinical margin to the surgical and HRCM margins at perpendicular 90° points around the lesion. For the lesion-based approach, a comparison was performed between the defect area estimated by HRCM imaging and the final surgical margin. We used paired *t* tests to assess differences in the estimated lesion area measured by HRCM and surgical excision. To assess differences in radial length between surgical margins and HRCM imaging, we used random-effects models. All analyses were performed using Stata SE software (version 14.1; StataCorp).

Results

Thirty-two patients consented to participate in the study and underwent imaging using HRCM-RV. Seven patients decided not to undergo surgery, and 3 decided to have treatment elsewhere. Nineteen LM and 4 LMM (median Breslow thickness, 0.37 mm; range, 0.30-1.05 mm) from 22 patients (12 men and 10 women) with a mean (SD) age of 69.0 (8.6) years (range, 46-83 years) contributed to the analysis. None of the LM or the LMM cases were upstaged after staged excision. Patient characteristics and imaging measurements are summarized in the eTable in the Supplement. The mean (SD) visualized clinical maximum diameter of the lesions was 1.86 (1.77) cm (range, 0.5-4.9 cm), and the mean (SD) area determined by clinical examination was 1.85 (1.77) cm^2 (range, 0.3-6.5 cm^2). The mean (SD) LM area based on HRCM was 3.9 (3.3) cm^2 (range, 1.1-16.0 cm^2). In all cases, HRCM confirmed the diagnosis of LM within the margin determined using dermoscopy or Wood lamp examination. In 40 of 92 quadrants (43.5%), HRCM identified LM beyond the clinical margin, extending a mean (SD) of 3.60 (2.60) mm (range, 0.50-11.00 mm). The mean (SD) surgical defect area estimated with HRCM was smaller than the surgical margin area (6.34 [4.02] vs 7.74 [5.28] cm^2; $P = .01$) (**Figure 3**).

A total of 92 pairs of radial measurements (4 per lesion, 1 per quadrant) were made from the clinical margin to the HRCM-estimated surgical margin. When evaluating the millimeters needed to be excised to achieve clearance, no differences were found in 58 of the 92 quadrants (63.0%) between the values estimated with HRCM and the observed surgical values. In 25 quadrants (27.2%), the defect was larger than the HRCM estimate by a mean of 2.24 mm (range, 1.00-6.00 mm), and in 9 quadrants (9.8%), HRCM overestimated the margin by a mean of 1.27 mm (range, 0.50-3.00 mm). Overall, after controlling for patient age, previous surgery, and clustering of observation within a given patient, surgical margins were a mean of 0.76 mm larger (95% CI, 0.67-0.84 mm; $P < .001$) than the HRCM estimate (**Figure 4**).

Table. RCM Features Considered as Positive for LM at the Edges

Source	RCM Features Positive for LM
Guitera et al,[13] 2017	
Major criteria	Nonedged dermal papillae
	Round, large pagetoid cells
Minor criteria	Nucleated cells in dermal papillae
	Atypical cells at DEJ
	Follicular localization of atypical cells
	Broadened honeycomb pattern (negative feature)
Champin et al,[15] 2014	Single large round cell or a large dendritic cell
Present study	Atypical dendritic cell (any size) continuing from the LM trailing edge

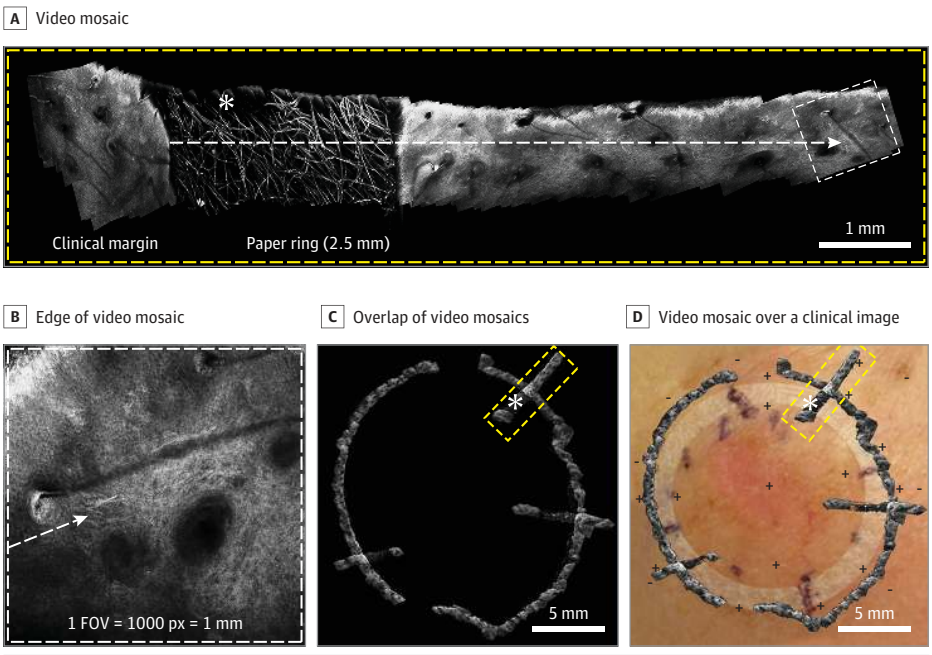
Abbreviations: DEJ, dermal-epidermal junction; LM, lentigo maligna; RCM, reflectance confocal microscopy.

Discussion

Although dermoscopy and Wood lamp examination have been used to guide LM excisions, in our study in approximately 40% of the quadrants, HRCM identified LM and/or LMM extending beyond the dermoscopic and Wood lamp margins. These results highlight that HRCM improves LM and LMM margin evaluation[12,14,15] and confirm the need for increased surgical margins to achieve clear margins.[4,24-26] We used an imaging approach that parallels the design of staged excision with radial histopathologic sectioning by imaging with HRCM radially from the center to the periphery. Our results suggest that HRCM-RV correlates with surgical defects after staged excision of LM and LMM, although the mean HRCM-RV estimates were smaller than the actual defect. This finding may be attributable to difficulty assessing LM edges in a background of sun-damaged skin, more precise margin delineation with RCM, or errors in margin estimation.

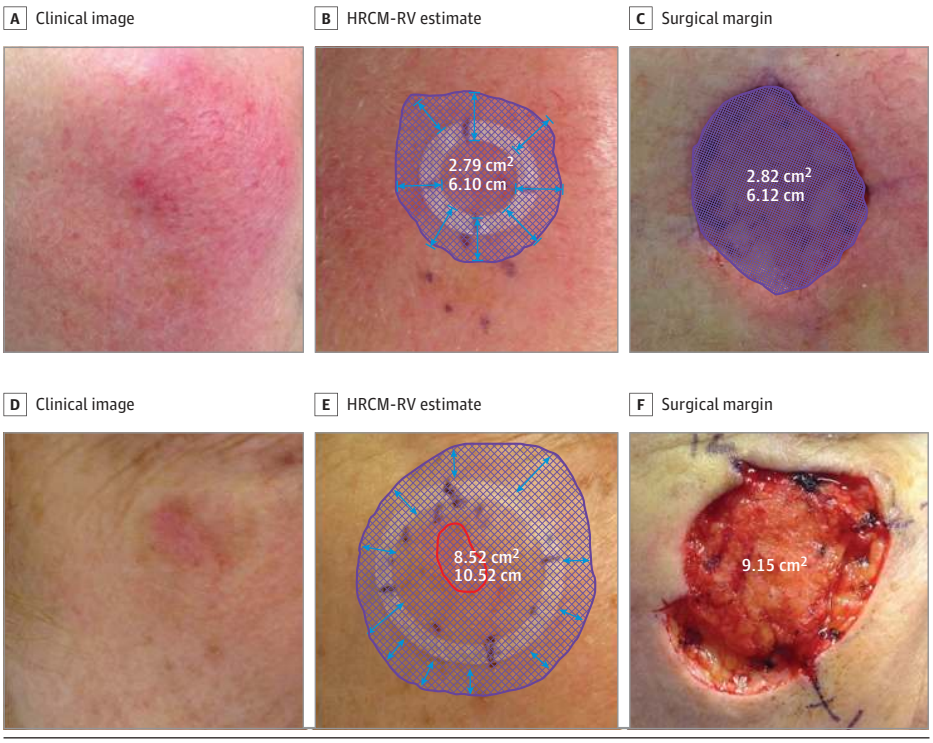
In this study, to consider an area at the lesion edges as positive for LM on HRCM, we used the features present in the original LM diagnostic algorithm,[13] the presence of isolated large atypical cells at the edges,[15] and the presence of smaller atypical dendritic cells continuing from the LM trailing edge (Table). Because atypical melanocytes can occur in healthy sun-exposed skin, distinguishing LM from sun-reactive melanocytic hyperplasia can be challenging on confocal and histopathologic evaluations.[27] Thus, by considering smaller atypical cells as being positive for LM, one could argue that numerous false-positive findings may occur because they could correspond to activated melanocytes that frequently occur in sun-exposed skin. However, our findings suggest that in most quadrants, HRCM estimates were equivalent to the final surgical margins or slightly smaller (mean of 0.76 mm), and we overcalled photodamage as LM only in 9 of 92 quadrants. The cases with bigger differences came from previously treated LM and LMM, suggesting that our approach performs better in nonrecurrent LM and LMM (eTable in the Supplement). Therefore, we believe that atypical melanocytes, regardless of their size if evolving from a fully fledged LM or LMM, should be con-

Figure 2. Margin Determination in Case 3 Using Handheld Reflectance Confocal Microscopy With Radial Video Mosaicing (HRCM-RV)



A, Video mosaic obtained from a video acquired at the 1-o'clock position from the center to the periphery (**Video**). Lentigo maligna (LM) extension was based on the paper ring rim width of 2.5 mm (asterisk), and the field of view (FOV) of the HRCM (dashed-edge box) is 1 mm, which equals 1000 pixels. B, The dashed-edge box from part A depicts a single FOV at the edge showing single dendritic cells continuing from the trailing edge of an LM at the 1-o'clock position. C, Digital overlap of 3 radial video mosaics obtained in 3 different lesion quadrants. D, A video mosaic map is digitally overlaid on a clinical image to estimate the surgical margin based on 3-mm histologic clearance. The yellow dashed-edge rectangles and asterisks correspond to the image in part A (C and D).

Figure 3. Examples of Lentigo Maligna Melanoma (LMM) and LM Imaged With Handheld Reflectance Confocal Microscopy With Radial Video Mosaicing (HRCM-RV) and Corresponding Surgical Defects After Staged Excision



Images A through C depict findings from case 2. A, Clinical image reveals an amelanotic, ill-defined LMM (Breslow thickness, 0.39 mm) with a clinical size of 0.26 cm^2. B, Estimated surgical margin after HRCM-RV finding of a 2.79-cm^2 defect. C, Final surgical defect was 2.82 cm^2 after 2 stages of excision. Images D through F depict findings from case 3. D, Clinical image reveals a hypomelanotic, ill-defined LM with a clinical size of 0.54 cm^2. E, Estimated surgical margin after HRCM-RV finding of an 8.52-cm^2 defect. F, Final surgical defect was 9.15 cm^2 after 3 stages of excision.

sidered as positive because they may reflect the trailing edge of an LM or LMM (Figure 2B).

Another explanation why our HRCM-estimated defects were smaller than the surgical defects may be that HRCM provides better margin delineation. Although our staged excision was conservative in terms of tissue sparing, several factors can influence the amount of tissue excised, including the thickness of the marking pen, the use of a magnifying glass to draw the marking, or where the incision is performed (inside or outside the skin marking).[23] These limitations are not present

when digitally diagramming the margins on an image. However, in quadrants where we did not identify LM outside the paper ring, we may have underestimated the margins by considering a quadrant negative if 2.5 mm of clearance were obtained. However, the clinical relevance of such small differences is unknown, and other margin-controlled surgical procedures may use less than 3 mm of histologic clearance for negative margins.[5] Therefore, if HRCM-RV was used to guide surgery, it could be the starting point of margin-controlled surgical procedures because it estimated a smaller and not a larger defect. This point is important because the presence of false-positive findings could lead to negative but wider-than-necessary margins.

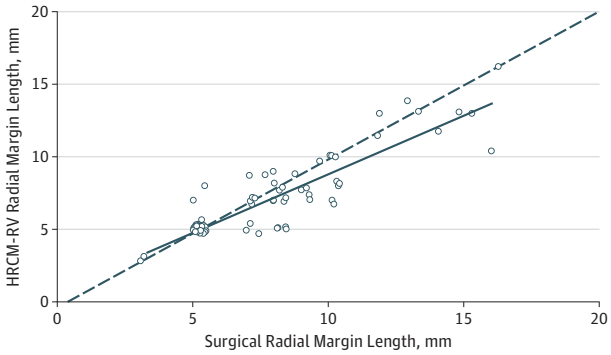
Limitations

This study has several challenges and limitations. We observed 9 false-positive quadrants, which corresponded to areas of photodamage with small dendritic cells on HRCM. The overestimation was a mean of 1.27 mm (range, 0.50-3.00 mm), which represents approximately an additional 6% to 37% margin relative to the real margin size. Previous studies have shown that variations owing to the surgical technique can range from 8% to 45% when margins from 2 to 10 mm are drawn on the skin.[23] In our study, overestimation occurred in fewer than 10% of quadrants and by a range that occurs in standard surgical procedures. To overcome variation in margin estimation owing to wound retraction after electrocoagulation, specimen shrinkage after formalin fixation,[28] or inaccuracy in the digital margin calculation, we performed 2 methods of image calibration, margin estimation, and video mosaic quadrant size estimation that, to our knowledge, have not been described in previous studies assessing LM and LMM margins.[7,8,14,15] However, additional studies involving multiple readers with varying levels of experience are needed to validate these results because our study included only 2 of us (M.C. and O.Y.) who acquired data and 1 (O.Y.) who analyzed the results.

Our approach combines 2 major innovations—adhesive rings and video mosaics—to guide navigation and calculate the surgical margins. A common challenge when using HRCM is identifying the exact area on the skin being imaged. Recently, adhesive rings have been used to define the area of interest when imaging with HRCM.[19,20,29] Adhesive rings are inexpensive, can be adapted to the shape of the lesion by slightly bending them or placing multiple rings outside the lesion margins, and can easily facilitate orientation because the fibers can be identified on HRCM, thus allowing a single operator to perform the image acquisition as opposed to the 2 operators needed with other LM mapping techniques that use HRCM.[15] We used commercially available paper rings with a rim width of 2.5 mm to calculate the surgical margins by combining them with video mosaicing; however, the diameter and rim width can be customized by cutting adhesive plastic,[29] thus permitting tailored HRCM navigation and margin estimation.

Another challenge when using HRCM is the absence of architectural information owing to lack of mosaicing capabilities in its native software. The ability to observe and examine architectural information in large fields of view is necessary, similar to that in pathologic analysis. For the newer HRCM with video acquisition, an algorithm has been developed to transform videos into video mosaics.[17,18,30] In the present study, we used RV to help delineate the LM and LMM margins. However, video mosaicing is not integrated in the HRCM software, and the postprocessing video takes approximately 10 minutes per 1 minute of each video. Nevertheless, each radial video is approximately 20 seconds long; thus, the whole HRCM-RV process is approximately 30 minutes long. Therefore, our next phase of research will focus on increasing the speed of postprocessing and integrating video mosaicing into the native HRCM software to obtain real-time video mosaics. This increase would allow faster margin estimation at the patient bedside and in the operating room. In the meantime, a simplified and less precise method to perform our radial imaging approach could be counting in real time the number of positive fields of view outside the paper ring while imaging radially from the center to the periphery.

Figure 4. Plot of the Paired Observations of the Surgical Margins and Estimated Handheld Reflectance Confocal Microscopy With Radial Video Mosaicing (HRCM-RV) Margins



Four measures were obtained from each sample, 1 from each lesion quadrant measured from the center of the lesion to the perceived margin. The dashed line represents the linear function (y = x), where surgical measures and HRCM-RV estimates are equal. The solid line represents the least squares fitted regression line for the association between the 2 measurement types. Hollow circles below the dotted line indicate observations for which the surgical margin was greater than the HRCM-RV margin; hollow circles on the dotted line, similar estimates; and hollow circles above the dotted line, observations for which HRCM-RV margins were larger than surgical margins. Observations were slightly offset to visualize overlapping data points. Overall, from our random-effects regression model and controlling for patient age and previous surgery, surgical margins were a mean of 0.76 mm (95% CI, 0.67-0.84 mm; $P < .001$) larger than the HRCM-RV estimates.

Conclusions

Our results suggest that HRCM-RV margin mapping correlates well with histologic findings and can estimate the subclinical extension and presurgical margins. Management of LM and LMM can benefit from noninvasive radial imaging, because evaluating an LM or LMM from the center toward the periphery illustrates its trailing edge as it dissipates into melanocytic hyperplasia[4]—a feature that can be difficult to contextualize when seen in an isolated sample, such as a scouting biopsy. Thus, we believe that our approach can result in

sparing of healthy tissue by reducing the number of biopsies in clinically uncertain areas. Furthermore, anticipation of the LM or LMM size and the estimated surgical defect may enable the clinician to decide the most adequate treatment, plan the surgical reconstruction in advance, and counsel the patient appropriately.

ARTICLE INFORMATION

Accepted for Publication: June 28, 2017.

Published Online: October 11, 2017. doi:10.1001/jamadermatol.2017.3114

Author Contributions: Drs Yélamos and Rossi had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Yélamos, Rajadhyaksha, Nehal, Rossi.
Acquisition, analysis, or interpretation of data: Yélamos, Cordova, Blank, Kose, Dusza, Lee, Nehal, Rossi.
Drafting of the manuscript: Yélamos, Dusza, Rajadhyaksha, Rossi.
Critical revision of the manuscript for important intellectual content: Cordova, Blank, Kose, Dusza, Lee, Rajadhyaksha, Nehal, Rossi.
Statistical analysis: Dusza.
Obtained funding: Yélamos, Rajadhyaksha.
Administrative, technical, or material support: Yélamos, Cordova, Blank, Kose, Rajadhyaksha.
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Conflict of Interest Disclosures: Dr Rajadhyaksha reports being a former employee of and owning equity in Caliber Imaging and Diagnostics (formerly Lucid Inc), the company that manufactures and sells the VivaScope confocal microscope. The VivaScope is the commercial version of an original laboratory prototype that he had developed at Massachusetts General Hospital, Harvard Medical School. No other disclosures were reported.

Funding/Support: This study was funded by grant R01EB020029 from the National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health (NIH); in part by Cancer Center Support grant P30 CA008748 from the National Cancer Institute, NIH; and by the Beca Excelencia Fundación Piel Sana (Dr Yélamos).

Role of the Funder/Sponsor: The sponsors had no role in the design and conduct of the study; in the collection, analysis, and interpretation of data; in the preparation, review, or approval of the manuscript; or in the decision to submit the manuscript for publication.

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