Diagnostic accuracy of pigmented labial macules by in vivo reflectance confocal microscopy and correlation among techniques

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Background: Pigmented labial macules (PLMs) are clinical, dermoscopic, and histopathologic challenges.

Objective: To describe and evaluate the utility of reflectance confocal microscopy (RCM) in PLMs and to establish a correlation between dermoscopy, RCM, histopathology, and immunohistochemistry.

Methods: Prospective study of PLMs from 4 tertiary referral dermatology centers. The study included 51 biopsy specimen-proven PLMs. Dermoscopic, RCM images, and histopathologic preparations were evaluated for malignant criteria. Diagnostic accuracy of RCM for melanoma diagnosis, RCM Lip Score previously reported, and κ values between techniques were calculated.

Results: Included were 5 melanomas and 46 benign PLMs. Dermoscopically, melanomas exhibited more frequently ≥3 colors and ≥3 structures. With RCM, pagetoid spreading, epithelial disarray, continuous proliferation of atypical cells around papillae, nonhomogeneously distributed papillae, marked cellular atypia, and a higher number of dendritic cells per papillae were more frequent in melanomas. The RCM Lip Score was significantly higher in malignant lesions. Good κ values were observed in most of the evaluated features. A perfect sensitivity and specificity was obtained combining dermoscopy and RCM.

Limitations: A low number of melanomas were obtained.

Conclusions: RCM improves lip melanoma diagnosis, and the RCM Lip Score represents a useful tool for the evaluation of a PLM. (J Am Acad Dermatol 2021;85:1151-60.)

Key words: dermoscopy; labial macule; melanoma; melanotic macule; reflectance confocal microscopy.

Many different lesions may present as a pigmented labial macule (PLM), such as melanotic macules, labial lentigo, melanocytic nevus, malignant melanoma (MM), and nonmelanocytic lesions (inflammatory, infectious, or foreign-body pigmentations).[1] Fortunately,

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malignancy in oral pigmented lesions is quite rare but tends to be aggressive, and surgical treatment can be complex and morbid.[2] Mucosal melanomas represent less than 5% of all MMs,[3,4] but they have a worse prognosis than cutaneous ones.[5] The presence of a homogeneous pigmentation, small size, and lack of progression are usually suggestive of benignity, whereas an atypical presentation with large size, variable colors, poorly demarcated borders, recent onset, or enlargement should raise suspicion of malignancy.[6]

Dermoscopy has greatly improved the diagnostic accuracy of skin tumors. In recent years, criteria have been proposed to aid in the evaluation of PLMs, but this remains confusing due to different terminologies used.[7-10] Dermoscopic monitoring or biopsy is usually recommended when the diagnosis of a benign PLM is not clear-cut.

Reflectance confocal microscopy (RCM) is a noninvasive technique useful in melanoma diagnosis. However, studies focused on its use in pigmented oral pathology remain limited.[11-16] The aim of our study was to describe the clinical, dermoscopic, and RCM findings of PLM, test the RCM Lip Score[16] previously described, evaluate the usefulness of RCM in the differential diagnosis of PLM, and correlate dermoscopy and RCM with histopathology.

CAPSULE SUMMARY

- Larger size, asymmetry, simultaneous skin-mucosal involvement, multicomponent pattern, and ≥3 colors should raise suspicion of mucosal melanoma in a pigmented labial macule.
- Reflectance confocal microscopy can improve mucosal melanoma diagnosis. The RCM Lip Score was significantly higher in malignant lesions and represents a useful tool in pigmented labial macules.

MATERIALS AND METHODS

PLMs from 23 patients attending Hospital del Mar (Barcelona) were included prospectively between September 1, 2015, and December 31, 2018. To enrich the population of mucosal melanomas, 28 cases from 3 other university hospitals (Sydney Melanoma Diagnostic Centre and Melanoma Institute Australia, Sydney, and Hospital Clínic, Barcelona) were retrospectively included. None of these cases were previously published and were consecutively selected after the publication of the RCM Lip Score.[16] The inclusion criteria used to recruit lesions in this study were pigmented and persistent labial lesions located completely or partially in the oral mucosa. Nodular lesions were excluded.

Clinical, dermoscopic, and RCM data

Clinical data and dermoscopic and RCM images were collected from all lesions (see the Supplementary material, available via Mendeley at https://doi.org/10.17632/gbdm38tjc4.1).

The multicenter International Dermoscopy Society study of pigmented lesions of the mucosa and mucocutaneous junction was used to analyze 28 dermoscopic criteria.[10] Dermoscopic and RCM criteria included in the evaluation process were selected based on the data available in the literature and from our preliminary observations. Two evaluators (I.G.M. and S.S., with 5 and 13 years of experience, respectively) assessed the presence or absence of predefined dermoscopic and RCM structures on stored images and calculated the RCM Lip Score,[16] blinded to dermoscopic images and histopathologic diagnosis.

The RCM Lip Score calculates a melanoma score based on 7 criteria. One major criterion (presence of dendritic or roundish pagetoid cells) is scored +2 points and 4 minor criteria (epidermal disarray, dishomogeneous distributed papillae, marked cellular atypia at the epithelial-chorion junction [ECJ], and interpapillar distribution of atypical cells in relationship with the papillae) are scored +1 point each. There are 2 protective (benign) criteria (regular honeycombed pattern and homogeneous distributed papillae) that receive a score of −1 point. An RCM Lip Score of ≥4 is suspicious for melanoma.

The specific diagnosis found by the readers, sensitivity, and specificity for melanoma diagnosis were calculated by I.G.M. (cases from Hospital del Mar) and by I.G.M. and S.S. (remaining cases) with pathology as the gold standard: firstly, after blinded evaluation of RCM images using the Lip Score,[16] and secondly, considering clinical and dermoscopic information. Clinical, dermoscopic, and confocal images were evaluated blinded to histopathology. Discrepant cases between readers were discussed together to get an agreement. In case of disagreement, a third experienced dermatologist was consulted to reach a consensus diagnosis. Skin biopsy specimens were obtained from all lesions. A partial biopsy of >3 mm at the most suspicious area was performed in 30 patients and total excision was done in 21 patients.

Immunohistochemical stain with melanocytic markers (melan-A, MiTF, or SOX10) were performed in 88% (45 of 51) of cases and CD1a in 84% (43 of 51) of cases.

Abbreviations used:

ECJ: epithelial-chorion junction
MM: malignant melanoma
PLM: pigmented labial macule
RCM: reflectance confocal microscopy

Histopathologic features were systematically evaluated (blinded to clinical information and histopathologic diagnosis) by 4 dermatopathologists (C.B., RM.P., L.A., and PM.F.). The previous histopathologic report was reviewed in 5 cases where the histopathologic specimen was not available for evaluation.

Statistical analysis

In bivariate analysis, dichotomous variables were evaluated by the χ^2 test. The Student *t* test was used to compare means between 2 groups. In multivariate analysis of dermoscopic and RCM features, a binary logistic regression was performed to investigate which dermoscopy and confocal features differentiate histologically confirmed MM from benign lesions. For correlations, the Cohen κ coefficient was calculated for each descriptor. Statistical evaluation was performed using SPSS 15.0 software (SPSS Inc, Chicago, IL).

RESULTS

Clinical findings

A total of 51 PLMs were included from 50 patients (38 women and 12 men), with a mean age of 54.5 (SD 18.1) years (range, 18-81 years). The lesions corresponded to 46 benign lesions (23 melanotic macules, 7 melanocytic hyperplasias, 5 lichenoid lesions, 3 solar lentigos, 2 ephelides, 2 pigmented actinic keratoses, 2 lentigo simplex lesions, 1 compound nevi, and 1 arteriovenous hemangioma) and 5 MMs. Lesions were mainly located in the inferior lip, both in the benign group (82.6%) and in melanomas (80%).

Clinically, melanomas showed a larger size compared with benign lesions (34 mm vs 8.75 mm, $P < .001$), asymmetry (100% vs 45.7%, $P = .037$), and affected simultaneously the mucosa and labial skin (60% vs 2.2%, $P = .002$).

Dermoscopic findings

Dermoscopic evaluation showed that malignant lesions presented more frequently with ≥3 colors (4 of 5 [80%] vs 12 of 41 [28.6%], $P = .043$) and ≥3 structures (4 of 5 [80%] vs 10 of 41 [24.4%], $P = .025$). Neither colors nor structures presented statistically significant differences between benign and malignant lesions (Table I).[17]

RCM findings

RCM criteria that showed statistically significant differences between the malignant and benign group are summarized in Table I. Examples of a malignant and a benign lesion are seen in Fig 1 and Supplemental eFig 1, respectively.

The RCM Lip Score[16] was calculated in all cases. The mean (SD) RCM Lip score was significantly higher in melanoma (3.8 [2.39]) than in benign lesions (−0.67 [1.75], $P < .001$). However, 2 melanomas had a score of <4 (false negative) by the RCM Lip Score. One lesion (Fig 2) had a Lip Score of 0 because most of the lesion was located on the cutaneous part of the lip, with only subtle mucosal changes, and the RCM Lip Score is not defined to evaluate cutaneous images. However, the readers correctly diagnosed it as malignant because the cutaneous part exhibited dermoscopic and RCM criteria described for lentigo maligna.[18-20] The second false negative case was an incipient lesion, where the excisional biopsy specimen revealed an atypical melanocytic proliferation with only focal progression to lentigo maligna, which may have been overlooked in the confocal assessment.

On the other hand, the RCM Lip Score only had 1 false positive, a melanotic macule (Fig 3) that obtained a Lip Score of 5 due to epidermal disarray, atypical dendritic pagetoid cells, dishomogeneously distributed papillae, and interpapillar distribution of atypical cells. The partial biopsy specimen showed an increase of basal melanocytes with scarce Langerhans cells in suprabasal layers that did not allow the nature of the dendritic intraepidermal cells observed in RCM to be accurately defined.

In the multivariate analysis, only the RCM Lip Score exhibited statistical significance ($P = .034$), with an odds ratio of 1.6 (95% confidence interval, 1.04-2.5).

Histopathologic and immunohistochemical findings

Histopathologic criteria that allowed us to differentiate benign PLMs from melanomas included melanocytic atypia (100% [4 of 4] vs 14.3% ([6 of 42], $P = .001$), basal melanocytic hyperplasia in hematoxylin and eosin (75% [3 of 4] vs 16.7% [7 of 42], $P = .028$), basal melanocytic hyperplasia with Melan-A stain (100% [2 of 2] vs 14.3% [3 of 21], $P = .04$) and SOX-10 (100% [3 of 3] vs 20.6% [7 of 34], $P = .015$), and pagetoid spreading of melanocytes (75% [3 of 4] vs 2.4% [1 of 42], $P = .001$). Some benign lesions exhibited melanocytic atypia (14.3% [6 of 42]), melanocytic hyperplasia in hematoxylin and eosin (16.7% [7 of 42]), and pagetoid spreading (2.4% [1 of 42]).

Table I. Frequencies of dermoscopic and reflectance confocal microscopy parameters

Features*	Malignant lesions (n = 5)	Benign lesions (n = 46)	*P* value (malignant vs benign lesions)
Clinical features			
Age, mean (SD), y	61.8 (23.6)	53.7 (18.2)	.364
Size, mm	34	8.75	<.001
Unique lesion	100 (5/5)	56.5 (26/46)	.066
Asymmetry	100 (5/5)	45.7 (21/46)	.037
Affects both mucosa and skin	60 (3/5)	2.2 (1/46)	.002
Dermoscopic features			
Colors, No.			
1 color	0 (0/5)	26.2 (11/42)	.322
2 colors	20 (1/5)	45.2 (19/42)	.377
≥3 colors	80 (4/5)	28.6 (12/42)	.04
Colors			
Light brown	100 (4/4)	97.6 (41/42)	>.99
Dark brown	75 (3/4)	41.5 (17/41)	.309
Black	50 (2/4)	7.3 (3/41)	.055
Blue	50 (2/4)	9.8 (4/41)	.08
Gray	50 (2/4)	41.5 (17/41)	>.99
White	50 (2/4)	9.8 (4/41)	.08
Red	25 (1/4)	9.8 (4/41)	.0387
Dermoscopic patterns, No.			
1 dermoscopic pattern	0 (0/4)	17.1 (7/41)	>.99
2 dermoscopic patterns	25 (1/4)	58.5 (24/41)	.309
Multicomponent pattern (≥3 patterns)	80 (4/5)	24.4 (10/41)	.025
Dermoscopic structures			
Lines (single or parallel)	75 (3/4)	51.2 (21/41)	0.611
Reticular lines	75 (3/4)	29.3 (12/41)	.101
Curved lines	50 (2/4)	24.4 (10/41)	.286
Circles	0 (0/4)	19.5 (8/41)	>.99
Globules/clods	25 (1/4)	9.8 (4/41)	.387
Dots	25 (1/4)	26.8 (11/41)	>.99
Structureless areas	100 (4/4)	51.2 (21/41)	.117
Blue-gray structureless areas	50 (2/4)	9.8 (4/41)	.08
Regression structures	0 (0/4)	7.3 (3/41)	>.99
Blue-white veil	25 (1/4)	0 (0/41)	.089
Ulceration	0 (0/4)	0 (0/41)	NI
Peppering	0 (0/4)	14.6 (6/41)	>.99
Atypical vascular pattern	25 (1/4)	4.9 (2/41)	.249
Reflectance confocal microscopy features			
Suprabasal epithelia			
Regular honeycombed pattern	40 (2/5)	73.9 (34/46)	.144
Atypical honeycomb	60 (3/5)	19.6 (9/46)	.078
Epithelial disarray	60 (3/5)	4.3 (2/46)	.005
Presence of pagetoid cells (large bright cells in epithelial layers)	100 (5/5)	10.9 (5/46)	<.001
Round pagetoid cells	80 (4/5)	4.3 (2/46)	<.001
Dendritic pagetoid cells	60 (3/5)	13 (6/46)	.033
Epithelial-chorion junction (ECJ)			
Ringed pattern of ECJ	100 (5/5)	60.9 (28/46)	.148
Polycyclic papillae (ECJ)	20 (1/5)	13 (6/46)	.538
Nonspecific pattern (ECJ)	20 (1/5)	39.1 (18/46)	.639
Trabecular or draped pattern (ECJ)	60 (3/5)	10.9 (5/46)	.023
Homogeneously distributed papillae	40 (2/5)	78.3 (36/46)	.098
Nonhomogeneously distributed papillae	80 (4/5)	15.2 (7/46)	.006
Edged papillae	80 (4/5)	63 (29/46)	.65
Papillae rimmed by highly reflective epithelial cells	40 (2/5)	32.6 (15/46)	>.99
Nonedged papillae	20 (1/5)	32.6 (15/46)	>.99

Continued

Table I. Cont'd

Features*	Malignant lesions (n = 5)	Benign lesions (n = 46)	*P* value (malignant vs benign lesions)
Nonvisible papillae	0 (0/5)	8.7 (4/46)	>.99
Absence of a hyper-reflective basal layer and presence 2-3 dendritic bright cells per papillae	60 (3/5)	47.8 (22/46)	.668
Continuous (lentiginous) proliferation of atypical enlarged bright cells around the papillae	60 (3/5)	2.2 (1/46)	.002
Basal dendritic cells (1 mm^2 × 1 mm^2), mean (SD) No.	23.6 (32.4)	15.4 (15.5)	.603
Cellular atypia at ECJ	80 (4/5)	47.8 (22/46)	.35
Marked cellular atypia at ECJ†	60 (3/5)	0 (0/46)	<.001
>3 atypical cells at the ECJ in 5 images (0.5 mm^2 × 0.5 mm^2)	40 (2/5)	34.8 (16/46)	>.99
Atypical‡ cells in 1 mm^2 × 1 mm^2, mean (SD), No	25.2 (26.3)	1.94 (2.8)	.119
Dendritic cells per papillae, mean (SD), maximum No.	8.6 (6.1)	2.3 (1.7)	.006
Atypical‡ dendritic cells at ECJ	60 (3/5)	54.3 (25/46)	>.99
Atypical‡ round cells at ECJ	60 (3/5)	2.2 (1/46)	.002
Interpapillar atypical cells	60 (3/5)	28.3 (13/46)	.309
Interpapillar dendritic cells in relationship with the papillae	80 (4/5)	39.1 (18/46)	.152
Network of dendritic cells and dendritic processes	20 (1/5)	21.7 (10/46)	>.99
Chorion			
Sparse cell nests in chorion	20 (1/5)	2.2 (1/46)	.188
Plump bright cells within the papillae	60 (3/5)	58.7 (27/46)	>.99
Plump bright cells and/or small bright particles in papillae	40 (2/5)	69.6 (32/46)	.318
Lip score			
Mean (SD)	3.8 (2.39)	−0.67 (1.75)	<.001
Median (range)	5 (0-6)	−2 (−2 to 5)	

NI, Not indicated.
*Categorical data are shown as number (%) and continuous data as indicated.
†Defined as numerous cells irregular in size, shape, and reflectivity, round to oval or stellate, occasionally with branching dendritic-like structures, and/or distributed throughout the lesion.[17]
‡Atypical cells are defined as cells with irregular size, shape and reflectivity.

Correlation between techniques

Dermoscopy–RCM. Blue-white veil in dermoscopy correlated well with sparse cell nests in chorion (κ = 0.656, $P < .001$), marked cellular atypia (κ = 0.483, $P < .001$), and continuous (lentiginous) proliferation of atypical enlarged bright cells (κ = 0.483, $P < .001$) in the RCM evaluation.

In addition, regression structures in dermoscopy correlated with nonvisible papillae (κ = 0.536, $P < .001$). Blue-gray structureless areas in dermoscopy correlated weakly with continuous (lentiginous) proliferation of atypical enlarged bright cells around the papillae (κ = 0.390, P = .005) in RCM.

RCM–histopathology and immunohistochemistry. Among RCM criteria, pagetoid cells in general and round pagetoid cells (but not dendritic pagetoid) both correlated well with pagetoid spreading in the histopathologic study (κ = 0.434, P = .001; and κ = 0.553, $P < .001$, respectively). Marked cellular atypia at the ECJ in RCM correlated with histopathologic melanocytic atypia (κ = 0.401, P = .001). Continuous proliferation of atypical enlarged bright cells around the papillae in RCM correlated with pagetoid spreading (κ = 0.452, P = .002).

Atypical round cells at ECJ in RCM correlated with melanocytic atypia (κ = 0.511, $P < .001$) and melanocytic hyperplasia in immunohistochemistry (κ = 0.425, $P < .001$).

Sparse cell nests in chorion observed by RCM correlated with melanocytic nests in histopathology (κ = 0.477, P = .001), and plump bright cells within the papillae correlated with inflammatory infiltrate (κ = 0.575, $P < .001$).

A high correlation was observed between the number of atypical cells in the ECJ in 1 mm^2 by RCM and the melanocytic density in the melanocytic stains (Pearson r = 0 .724, $P < .001$). In addition, the maximum number of dendritic cells per papillae in RCM correlated well with the melanocytic density in the melanocytic stains (Pearson r = 0.606, $P < .001$). However, no correlation was observed between basal or papillary dendritic cells in RCM and Langerhans cells by immunohistochemistry.

Specific diagnosis, sensitivity, and specificity

The accuracy of dermoscopy and RCM for melanoma diagnosis and specific diagnosis were calculated blinded to histopathologic diagnosis (Table II).

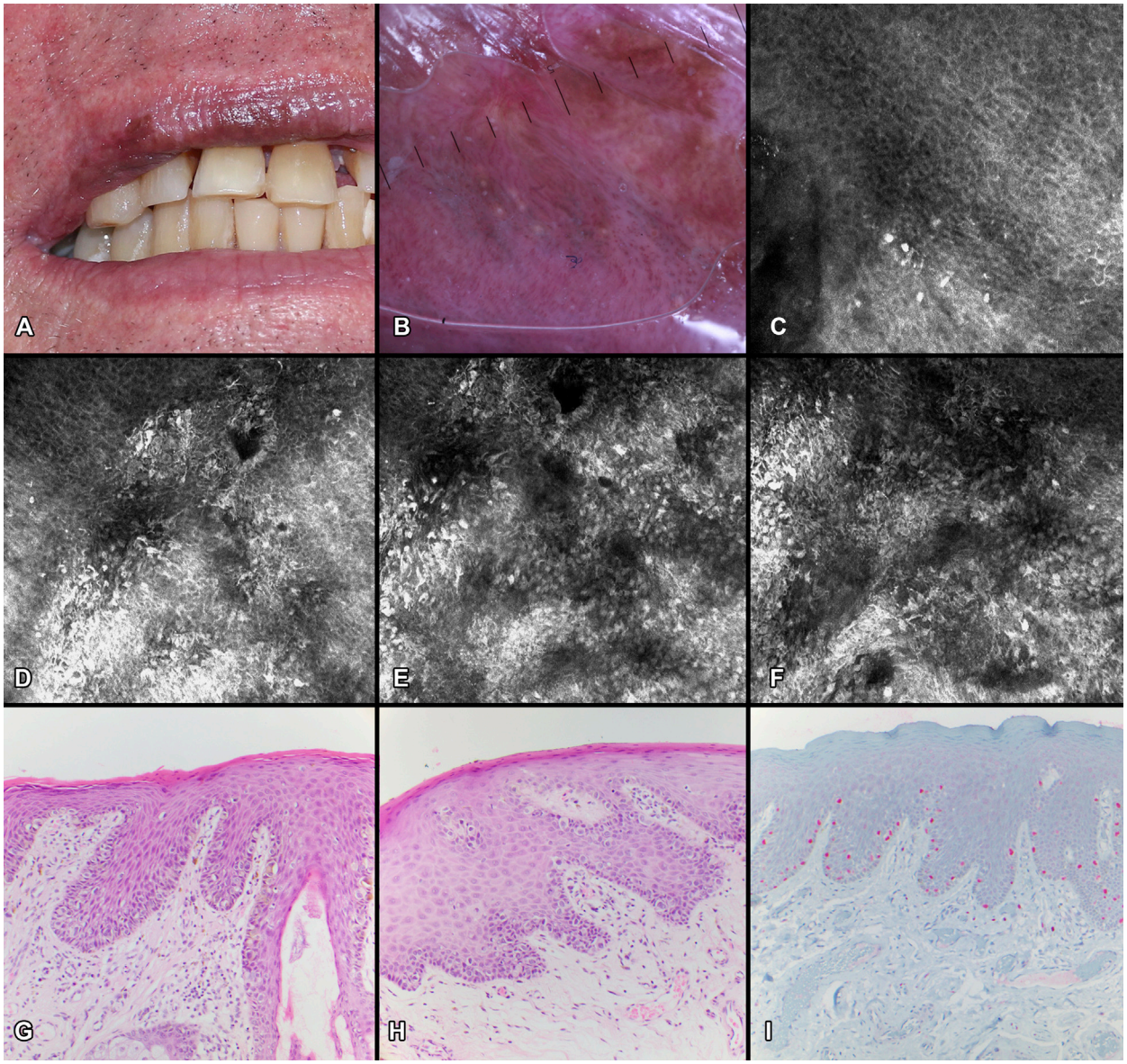

Fig 1. In situ malignant melanoma, lentigo maligna type (true positive of the reflectance confocal microscopy [RCM] Lip Score). (**A**) Asymmetric pigmented labial macule (35 mm × 10 mm) on the superior lip in a man in his 80s. (**B**) Dermoscopic image reveals a lesion with multicomponent pattern (parallel lines, reticular lines, and structureless areas), ≥3 colors, and irregular borders. RCM image (0.5 mm × 0.5 mm) shows (**C**) detail of round pagetoid cells, (**D**) continuous (lentiginous) proliferation of atypical enlarged bright cells in the papillae and sheets of atypical cells at the epithelial-chorion junction, and (**E, F**) nonedged papillae and sheets of atypical cells at the epithelial-chorion junction. Note roundish and stellate atypical cells. (**G**) Biopsy specimen revealed an atypical proliferation of melanocytes in the basal layers of epidermis with extension along follicular structures suggestive of lentigo maligna (hematoxylin and eosin, cutaneous component; original magnification: ×200. (**H**) Atypical proliferation of melanocytes in the epithelium (hematoxylin and eosin, mucosal component; original magnification: ×200). (**I**) SOX10 staining, in the mucosal component, labeling the nucleus of melanocytes.

DISCUSSION

PLMs comprise a heterogeneous group of entities with different clinical relevance and management, varying from inflammatory conditions to skin cancer. To establish a definite diagnosis, biopsy specimens of the oral mucosa are frequently required due to

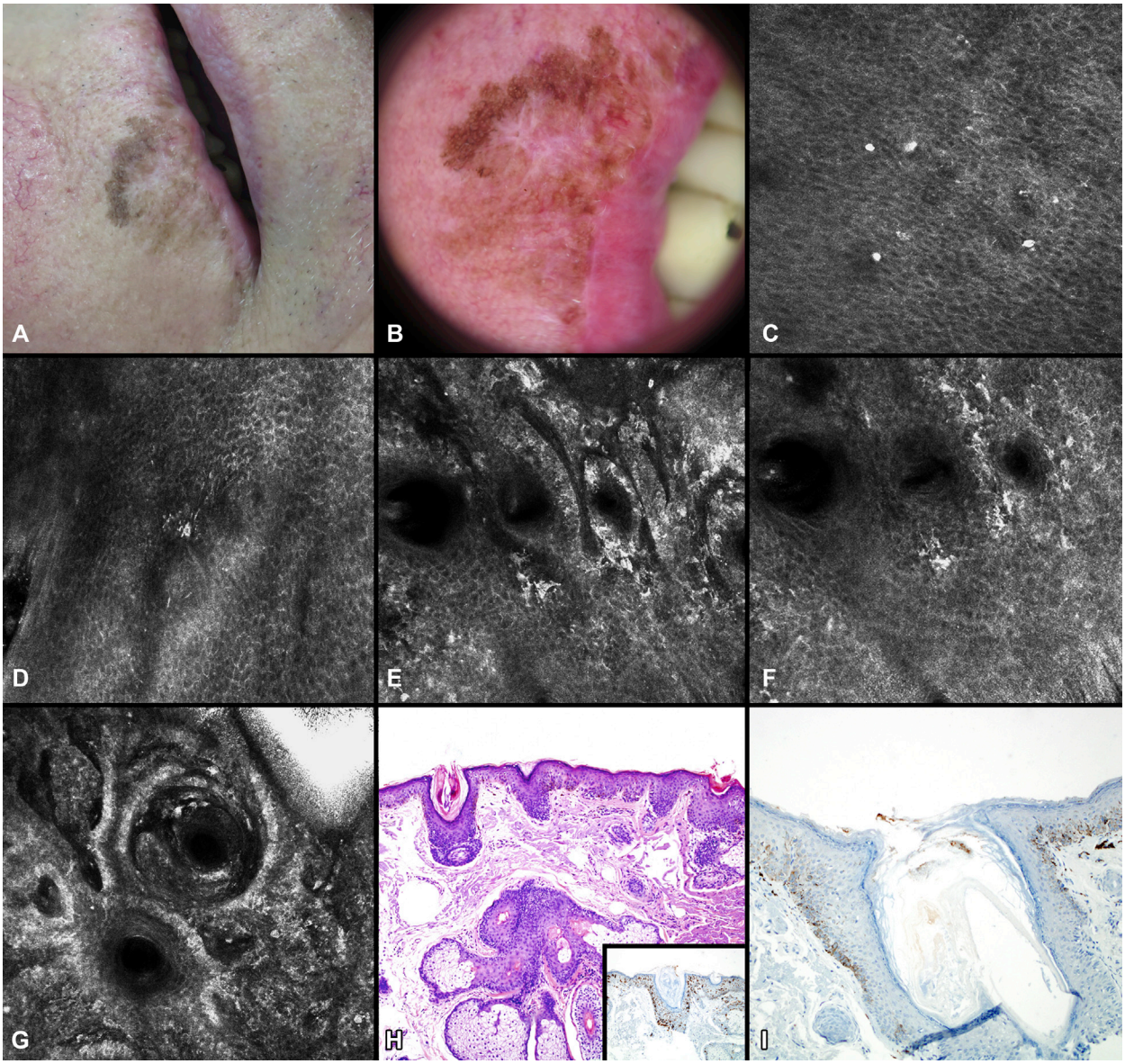

Fig 2. Lentigo maligna (false negative of the reflectance confocal microscopy [RCM] Lip Score in the mucosal evaluation). (**A**) Asymmetric pigmented labial macule (30 mm × 21 mm) affecting predominantly the cutaneous part of the inferior lip in a man in his 70s. (**B**) Dermoscopic view shows light and dark-brown pigmented pseudonetwork, asymmetric pigmented follicular openings, and some double circle (circle in circle) structures. Note the whitish scar of a previous biopsy in the center of the lesion. RCM image (0.75 mm × 0.75 mm) on the cutaneous part of the lesion shows (**C**) detail of round pagetoid cells and (**D**) detail of a dendritic pagetoid cell. (**E**) RCM image (0.75 mm × 0.75 mm) at the dermal-epidermal junction shows >3 atypical dendritic cells. (**F**) RCM image (0.75 mm × 0.75 mm) shows follicular localization of atypical dendritic cells. (**G**) RCM image (0.75 mm × 0.75 mm) shows >3 atypical cells at the dermal-epidermal junction, some with follicular distribution. (**H**) Biopsy specimen revealed an atypical proliferation of melanocytes in an atrophic epidermis with extension along follicular structures compatible with melanoma (lentigo maligna type). Elastosis in the upper dermis (hematoxylin and eosin stain, original magnification: ×200). Inset: abundant Langerhans cells associated in the epidermis (CD1a stain). (**I**) Melan-A staining (original magnification: ×200), labeling the atypical proliferation of melanocytes (cytoplasm) in the epidermis and adnexa.

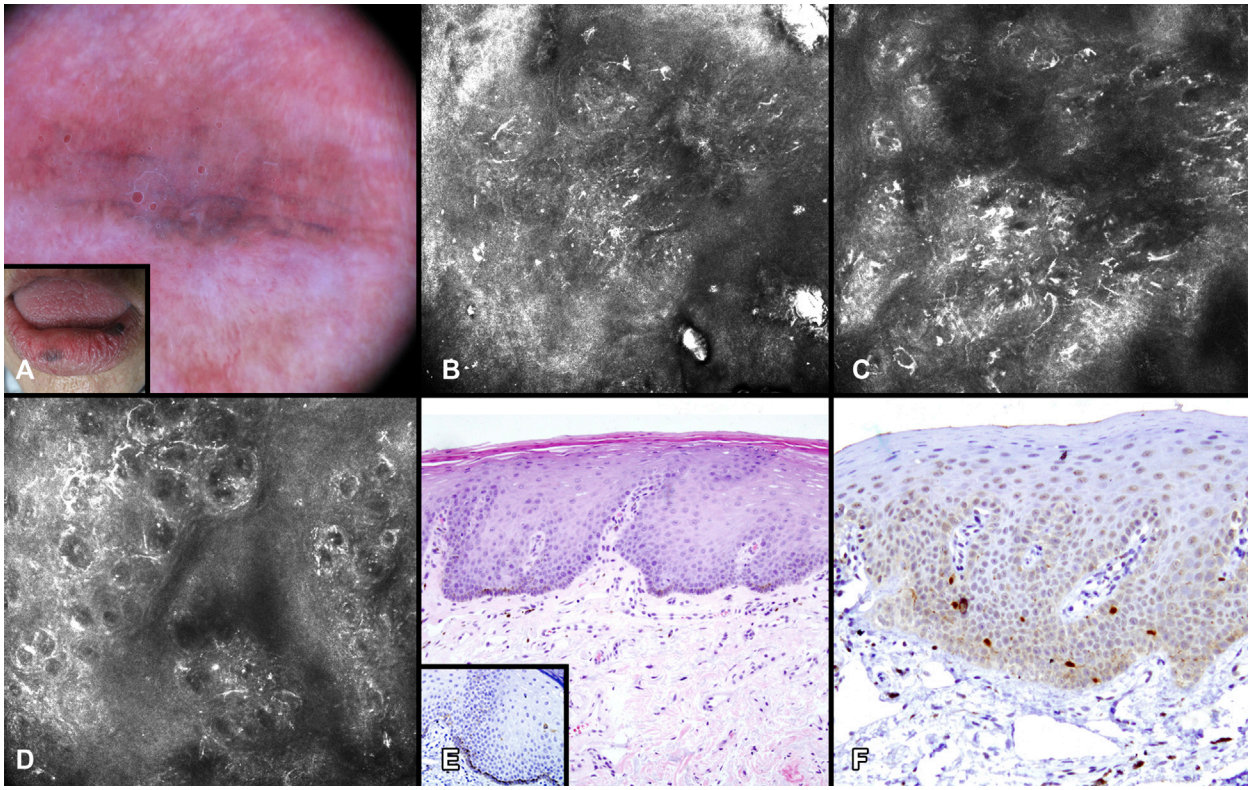

Fig 3. Melanotic macule (false positive of the Reflectance Confocal Microscopy [RCM] Lip Score). (**A**) Symmetric pigmented labial macule (10 mm × 7 mm) on the inferior lip in a woman in her 80s. Dermoscopic view of the medial lesion shows light and dark-brown lines and grayish structureless zones on the center. Inset: Clinical view. (**B**) RCM image (0.75 mm × 0.75 mm) shows detail of pagetoid dendritic cells in higher layers of the epithelium. (**C**) RCM image (0.75 mm × 0.75 mm) at the epithelial-chorion junction shows >3 atypical dendritic cells. (**D**) RCM image (0.75 mm × 0.75 mm) shows detail of nonhomogeneously distributed papillae around the lesion and the presence of 2 to 3 dendritic bright cells per papillae. (**E**) Biopsy specimen revealed a melanotic macule (hematoxylin and eosin stain, original magnification: ×200). Inset: Detail of some basal dendritic Langerhans cells with scarce intraepithelial cells (CD1a stain). (**F**) Activated melanocytes in the basal layers of the epithelium (SOX10 stain).

lack of clinically and dermoscopically specific criteria of oral MM, specially in early phases.

In this study, we found that larger lesion size, asymmetry, and the involvement of the skin and mucosa at the same time confer more risk of malignancy. Of the 5 MMs, 3 (60%) affected both skin and mucosa, but only 1 benign lesion (2.2%) affected both. This benign lesion was histopathologically diagnosed as an atypical melanocytic hyperplasia based on two 4-mm punch biopsy specimens and a follow-up time of 12 months, which might not be enough to completely discard the diagnosis of MM.

In our opinion, all mucosal biopsies with melanocytic hyperplasia should be followed-up, rebiopsied, or completely excised due to the risk of being an early melanoma, because melanotic macules by definition should exhibit a normal number of melanocytes or only a slight increase.[21,22] In our study, the 7 melanocytic hyperplasias (5 with atypia) included have been monitored for 6 to 18 months, and all of them were biopsied at least once with benign results. In this scenario, close monitoring by RCM could avoid biopsies.

Dermoscopically, we observed that any color or dermoscopic structure might be present in either benign or malignant lesions; however, the presence of a multicomponent pattern of ≥3 structures or ≥3 colors in a lesion should raise suspicion of malignancy. These results are in agreement with previous articles that also observed asymmetry, a multicomponent pattern, and multiple colors in malignant lesions.[7,9,10]

Table II. Specific diagnosis, sensitivity, and specificity of each technique

		Melanoma diagnosis (benign/malignant), %		
Technique	Specific diagnosis, %	Specific diagnosis	Sensitivity for diagnosis	Specificity for diagnosis
Cx-Dx	53.2	89.4	80	90.5
bRCM	47.1	94.1	60	97.8
RCM	62.7	100	100	100

bRCM, Reflectance confocal microscopy evaluation according to RCM Lip Score blinded to clinical and dermoscopic images; *Cx-Dx*, clinical-dermoscopic evaluation; *RCM*, reflectance confocal microscopy evaluation aided by clinical-dermoscopic images.

Regarding the RCM evaluation, roundish cells (basal or suprabasal, or both) and a higher density of dendritic cells per papillae were clues to malignancy, in agreement with previous studies.[11,13,15] Moreover, we found that the presence of roundish or dendritic pagetoid cells, or both, the continuous proliferation of melanocytes around the dermal papillae, marked cellular atypia at the ECJ, epithelial disarray, the presence of atypical round cells at the ECJ, and the nonhomogeneously distributed papillae are also features suggesting malignancy, as observed by Uribe et al.[16]

As already pointed out in previous studies,[12,15] we observed some "dendritic type" melanotic macules with an irregular dendritic pattern (melanotic macules with RCM features overlapping with MM), which represented challenging cases in the RCM evaluation (Fig 3). In some cases, those dendritic cells observed in RCM correlated well with Langerhans cells in histopathology (Supplemental eFig 1).

We did not, however, find statistical correlation between dendritic cells in RCM and Langerhans cells in immunohistochemistry, which might be due to the difficulties in differentiating Langerhans cells and dendritic melanocytes in the RCM evaluation and the difficulty of counting the same exact area in RCM and histopathology, also considering that both techniques are evaluated in different planes. Otherwise, we could demonstrate good correlation between the number of atypical cells in the ECJ by RCM and basal melanocytic density in the melanocytic stains.

We observed that the clinical-dermoscopic evaluation had a notable sensitivity and specificity when facing PLMs; however, RCM allows a great opportunity to improve the correct diagnosis. Clinical-dermoscopic and blinded RCM evaluation of PLMs had an elevated rate of misdiagnosis, and blinded RCM carried lower sensitivity than the clinical-dermoscopic examination. Remarkably, RCM after clinical and dermoscopic assessments obtained the greatest diagnostic accuracy, sensitivity, and specificity.

Unfortunately, the RCM accuracy for a precise diagnosis remained slightly low (62.7%). This may be explained by the difficulty to guess the diagnosis among the wide range of existing benign entities, especially when they only differ by the presence of scarce inflammatory cells on the ECJ, subtle pigmentation of the rete ridges, or dermal melanophages. These cases are also difficult to resolve by histopathology (gold standard technique) into precise diagnoses (for instance, to differentiate a melanotic macule from a residual dermal pigmentation due to a lichenoid process), and sometimes, pathologists simply describe the process, highlighting the absence of malignancy, without giving an accurate/definitive diagnosis, such as melanocytic hyperplasia or dermal pigmentation. On the other hand, in our experience, RCM also helps to identify the most atypical areas to biopsy from lesions that might be heterogeneous on histopathology.

In our study, the RCM Lip score[16] was significantly higher in malignant lesions (3.8 [SD, 2.39]) than in benign lesions (−0.67 [SD, 1.75]), $P < .001$). In the multivariate analysis, the RCM Lip Score was the only criteria exhibiting statistical significance (95% confidence interval, 1.04-2.5; $P = .034$). This provides further evidence for the value of the RCM Lip Score as a useful tool in PLMs, but it is not devoid of possible errors without clinical and dermoscopic information. Those PLMs with mucosal and skin involvement should be assessed carefully with cutaneous and mucosal RCM criteria.

A limitation of the study is that definite diagnosis was sometimes established according to a 4-mm punch biopsy evaluation chosen by RCM findings. This small sample size may have caused misdiagnosis if the biopsy specimen was not representative of the entire lesion, especially in cases with borderline findings on histopathology. In relation to this, 7 lesions with a diagnosis of melanocytic hyperplasia were included in the study as benign lesions. Although this is an "uncertain" diagnosis, it is a common situation that dermatologists face in clinical practice, which will need follow-up or new biopsy specimens to finally rule out malignancy.

We should remark that the design of this study might have led to high sensitivity and specificity that is not always achieved in real-world dermatology.

Unfortunately, owing to the low number of malignant lesions in the study despite the cooperation of 4 tertiary referral centers, the RCM Lip Score was the only significant criteria in the multivariate analysis.

CONCLUSIONS

Clinical and dermoscopic evaluations are essential to assess PLM. However, in those lesions with suspicious (bigger size, asymmetry, cutaneous and mucosal lip involvement, multicomponent pattern, and ≥3 colors) or indeterminate features, RCM should be considered because it has the potential to improve diagnostic accuracy as a complementary tool to the clinical and dermoscopic evaluation. However, on those lesions where even the histopathologic evaluation is not certain, careful follow-up (ideally with serial photography or new RCM evaluations) or repeated biopsies, or both, are mandatory to ensure the correct diagnosis.

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