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2 **Title:** Diagnostic accuracy of pigmented labial macules by in vivo reflectance confocal
3 microscopy and correlation among techniques

4 Ignacio Gómez-Martín, MD¹, Helena Collgros, MD², Peter M. Ferguson, MD, PhD³,
5 Alicia Barreiro, MD⁴, Pascale Guitera, MD, PhD^{2,5}, Evelyn Andrades-López, BS⁶,
6 Susana Puig, MD, PhD^{4,7}, Ramon M. Pujol, MD, PhD^{1,6}, Carlos Barranco, MD⁸, Llúcia
7 Alòs, MD, PhD⁹, Sonia Segura, MD, PhD^{1,6}

- 8 1. Department of Dermatology, Hospital del Mar-Parc de Salut Mar, Departament
9 de Medicina de la UAB, Universitat Autònoma de Barcelona, Barcelona, Spain
10 2. Sydney Melanoma Diagnostic Centre, Royal Prince Alfred Hospital, Sydney
11 3. Department of Tissue Pathology and Diagnostic Oncology, Royal Prince Alfred
12 Hospital, Sydney
13 4. Department of Dermatology, Hospital Clínic-IDIBAPS, University of
14 Barcelona, Barcelona, Spain
15 5. Melanoma Institute Australia, Sydney and Central Clinical School, Sydney
16 University
17 6. Institut Mar d'Investigacions Mèdiques (IMIM), Barcelona
18 7. CIBER de Enfermedades Raras, Instituto de Salud Carlos III, Barcelona, Spain
19 8. Department of Pathology, Hospital del Mar-Parc de Salut Mar, Barcelona
20 9. Department of Pathology, Hospital Clínic. Universitat de Barcelona, Barcelona

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22

23 **Corresponding author:**

24 Ignacio Gómez-Martín, MD

25 Department of Dermatology, Hospital del Mar-Parc de Salut Mar, Departament de
26 Medicina de la UAB, Universitat Autònoma de Barcelona. Barcelona, Spain
27 Passeig Marítim 25-29, 08003, Barcelona, Spain
28 Telephone: +34932483380 Fax: +34932483328
29 E-mail: nachgm@gmail.com

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Abstract

Background: Pigmented labial macules (PLM) are clinical, dermoscopic and histopathological challenges. **Objective:** To describe and evaluate the utility of reflectance confocal microscopy (RCM) in PLM and to establish a correlation between dermoscopy, RCM, histopathology and immunohistochemistry.

Methods: Prospective study of PLM from four tertiary referral centers of Dermatology. Fifty-one (biopsy proven) PLM were included in the study. Dermoscopic, RCM images and histopathological preparations were evaluated for malignant criteria. Diagnostic accuracy of RCM for melanoma diagnosis, RCM Lip Score previously reported and kappa values between techniques were calculated. **Results:** Five melanomas and 46 benign PLM were included. Dermoscopically, melanomas exhibited more frequently ≥ 3 colors and ≥ 3 structures. With RCM, pagetoid spreading, epithelial disarray, continuous proliferation of atypical cells around papillae, non-homogeneously distributed papillae, marked cellular atypia and higher number of dendritic cells per papillae were more frequent in melanomas. The RCM Lip Score was significantly higher in malignant lesions. Good kappa 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.

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. RCM Lip Score was significantly higher in malignant lesions and represents a useful tool in pigmented labial macules.

Introduction

Many different lesions may present as a pigmented labial macule (PLM) such as melanotic macules, labial lentigo, melanocytic nevus, malignant melanoma (MM) and non-melanocytic lesions (inflammatory, infectious or foreign-body pigmentations).¹ Fortunately, 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 MM,^{3,4} but they have 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 diagnostic accuracy of skin tumours. In recent years, criteria have been proposed to aid in the evaluation of PLM, 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 Lip score¹⁶ previously described, evaluate the usefulness of RCM in the differential diagnosis of PLM and to correlate dermoscopy and RCM with histopathology.

Materials & methods: PLM from 23 patients attending Hospital del Mar (Barcelona) were included prospectively between 1st September, 2015 and December 31st, 2018. Twenty-eight cases from three other university hospitals were retrospectively included (Sydney Melanoma Diagnostic Centre and Melanoma Institute Australia, Sydney and Hospital Clínic, Barcelona) to enrich the population of mucosal melanomas. None of these cases were previously published and they 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 data and dermoscopic and RCM images were collected from all lesions (see Supplementary material). Twenty-eight dermoscopic criteria were analyzed according to the multicenter International Dermoscopy Society study of pigmented lesions of the mucosa and mucocutaneous junction.¹⁰ Dermoscopic and RCM criteria included in the evaluation process were selected based on the data available in the literature and in 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 pre-defined dermoscopic and RCM structures on stored images and calculated the RCM Lip Score¹⁶, 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 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 gold standard, firstly after blinded evaluation of RCM images using the Lip Score¹⁶ 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 $>3\text{mm}$ at the most suspicious area was performed in 30 cases and total excision was done in 21 cases.

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

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

Statistical analysis

In bivariate analysis, dichotomous variables were evaluated by chi-square test. To compare means between two groups t-student test was used. 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 kappa coefficient was calculated for each descriptor. Statistical evaluation was performed using SPSS statistical software for Windows, version 15.0 (SPSS Inc).

Results:

Clinical findings

A total of 51 PLM were included from 50 patients (38 women; 12 men), ages ranging from 18 to 81 years (mean (SD): 54.5 (18.1)). The lesions corresponded to 46 benign lesions (23 melanotic macules, 7 melanocytic hyperplasia, 5 lichenoid lesions, 3 solar lentigo, 2 ephelis, 2 pigmented actinic keratosis, 2 lentigo simplex, 1 compound nevi, 1 arteriovenous hemangioma) and 5 MM.

Lesions were mainly located in the inferior lip, both in benign group (82.6%) and in melanomas (80%).

Clinically, melanomas showed a larger size in comparison with benign lesions (34mm vs 8.75mm, $p < 0.001$), asymmetry (100% vs 45.7%; $p = 0.037$) and affected simultaneously the mucosa and labial skin (60% vs 2.2%; $p = 0.002$).

Dermoscopic findings

Dermoscopic evaluation showed that malignant lesions presented more frequently ≥ 3 colors (4/5 (80%) vs 12/41 (28.6%); $p = 0.043$) and ≥ 3 structures (4/5 (80%) vs 10/41 (24.4%); $p = 0.025$).

Neither colors nor structures presented statistically significant differences between benign and malignant lesions (Table 1).

RCM findings

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

The RCM Lip Score¹⁶ 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)); $p < 0.001$. However, two melanomas had a score < 4 (false negative (FN)) 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, it was correctly diagnosed as malignant by the readers since the cutaneous part exhibited dermoscopic and RCM criteria described for lentigo maligna (LM).¹⁷⁻¹⁹ The second FN case was an incipient lesion, where the excisional biopsy revealed an atypical melanocytic proliferation with only focal progression to LM, that may have been overlooked in the confocal assessment. On the other hand, the RCM Lip Score only had one false positive (FP), 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 showed an increase of basal melanocytes with scarce Langerhans cells in suprabasal layers that did not allow to define accurately the nature of the dendritic intraepidermal cells observed in RCM.

In the multivariate analysis, only the RCM Lip Score exhibited statistical significance ($p = 0.034$, Conf. Interval: 1.04-2.5) with an Odds Ratio of 1.6.

Histopathologic and Immunohistochemical Findings

Histopathologic criteria that allowed us to differentiate benign PLM from melanomas included melanocytic atypia (100% (4/4) vs 14.3% (6/42), $p=0.001$), basal melanocytic hyperplasia in H/E (75% (3/4) vs 16.7% (7/42), $p=0.028$), basal melanocytic hyperplasia with Melan-A stain (100% (2/2) vs 14.3% (3/21), $p=0.04$) and SOX-10 (100% (3/3) vs 20.6% (7/34), $p=0.015$) and pagetoid spreading of melanocytes (75% (3/4) vs 2.4% (1/42), $p=0.001$). Some benign lesions exhibited melanocytic atypia (14.3%, 6/42), melanocytic hyperplasia in H/E (16.7%, 7/42) and pagetoid spreading (2.4%, 1/42).

Correlation between techniques

Dermoscopy-RCM

Blue-white veil in dermoscopy correlated well with sparse cell nests in chorion ($k=0.656$, $P<.001$), marked cellular atypia ($k=0.483$, $P<.001$) and continuous (lentiginous) proliferation of atypical enlarged bright cells ($k=0.483$, $P<.001$) in RCM evaluation. In addition, regression structures in dermoscopy correlated with nonvisible papillae ($k=0.536$, $P<.001$). Blue-gray structureless areas in dermoscopy correlated weakly with continuous (lentiginous) proliferation of atypical enlarged bright cells around the papillae ($k=0.390$, $P=.005$) in RCM.

RCM-Histopathology and Immunohistochemistry

Among RCM criteria, both pagetoid cells in general and round pagetoid cells (but not dendritic pagetoid) correlated well with pagetoid spreading in the histopathologic study ($k=0.434$, $P=.001$ and $k=0.553$, $P<.001$, respectively). Marked cellular atypia at ECJ in RCM correlated with histopathologic melanocytic atypia ($k=0.401$, $P=.001$).

Continuous proliferation of atypical enlarged bright cells around the papillae in RCM correlated with pagetoid spreading ($k=0.452$, $P=.002$). Atypical round cells at ECJ in RCM correlated with melanocytic atypia ($k=0.511$, $P<.001$) and melanocytic hyperplasia in immunohistochemistry ($k=0.425$, $P<.001$). Sparse cell nests in chorion observed by RCM correlated with melanocytic nests in histopathology ($k=0.477$, $P=.001$) and plump bright cells within the papillae correlated with inflammatory infiltrate ($k=0.575$, $P<.001$). A high correlation was observed between the number of atypical cells in the ECJ in 1mm^2 by RCM and the melanocytic density in the melanocytic stains (Pearson= 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= 0.606 , $p<.001$). However, no correlation was observed between basal or papillar 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 histopathological diagnosis (Table 2).

Discussion

PLM comprise a heterogeneous group of entities with different clinical relevance and management, varying from inflammatory conditions to skin cancer. In order to establish a definite diagnosis, biopsies of the oral mucosa are frequently required due to lack of clinical and dermoscopic 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. The 60% (3/5) of

MM affected both skin and mucosa, but only 1 benign lesion (2.2%). This benign lesion was histopathologically diagnosed as an atypical melanocytic hyperplasia based on two 4-mm punch biopsies and a follow-up time of 12 months, maybe not enough to discard the diagnosis of MM completely.

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, since melanotic macules by definition should exhibit a normal number of melanocytes or only a slight increase.^{20,21} In our study, the 7 melanocytic hyperplasias (5 with atypia) included have been followed-up for 6-18 months, all of them 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 (≥ 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, multicomponent pattern and multiple colors in malignant lesions.^{7,9,10}

Regarding RCM evaluation, roundish cells (basal and/or suprabasal) 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 and/or dendritic pagetoid cells, the continuous proliferation of melanocytes around DP, marked cellular atypia at ECJ, epithelial disarray, the presence of atypical round cells at ECJ and the non-homogeneously 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 RCM evaluation (Fig.3). In some cases those dendritic cells observed in RCM correlated well with Langerhans cells in histopathology (eFig.1).

However, we did not found statistical correlation between dendritic cells in RCM and Langerhans cells in immunohistochemistry, maybe due to the difficulties to differentiate 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 clinical-dermoscopic evaluation had a notable sensitivity and specificity when facing PLM, however, RCM allows a great opportunity to improve the correct diagnosis. Clinical-dermoscopic and bRCM evaluation of PLM had an elevated rate of misdiagnosis, and bRCM carried lower sensitivity than 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 score¹⁶ was significantly higher in malignant lesions (3.8+/-2.39) than in benign lesions (-0.67+/-1.75); $p < 0.001$. In the multivariate analysis, the RCM Lip Score was the only criteria exhibiting statistical significance ($p = 0.034$, Conf. Interval: 1.04-2.5). This provides further evidence for the value of the RCM Lip Score as a useful tool in PLM, but it is not devoid of possible errors without clinical and dermoscopic information. Those PLM 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 was not representative of the entire lesion, especially in cases with borderline findings on histopathology. In relation to this, lesions with a diagnosis of melanocytic hyperplasia were included in the study as benign lesions. Although it is an “uncertain” diagnosis, it is a common situation that dermatologists face in clinical practice, which will need follow-up or new biopsies 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, due to the low number of malignant lesions in the study despite the cooperation of four tertiary referral centres, 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, since it has the potential to improve diagnostic accuracy, as a complementary tool to 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) and/or repeated biopsies are mandatory to ensure the correct diagnosis.

References

1. Buchner A, Merrell PW, Carpenter WM. Relative frequency of solitary melanocytic lesions of the oral mucosa. *J Oral Pathol Med.* 2004;33(9):550-557.
2. Moretti A, Vitullo F, Augurio A et al. Surgical management of lip cancer. *Acta Otorhinolaryngol Ital.* 2011;31(1):5-10.
3. Wick MR. Prognostic factors for cutaneous melanoma. *Am J Clin Pathol.* 1998;110(6):713-718.
4. Saida T, Kawachi S, Takata M et al. Histopathologic characteristics of malignant melanoma affecting mucous membranes: a unifying concept of histogenesis. *Pathology.* 2004;36(5):404-413.
5. Froix AJ, Salti GI. Primary malignant melanoma of the lip. *J Surg Oncol.* 2003;84(1):7-9
6. Sison-Torre EQ, Ackerman AB. Melanosis of the vulva: a clinical simulator of malignant melanoma. *Am J Dermatopathol.* 1985;7(Suppl):51-60.
7. Puig S, Malvehy J, eds. *Principles of Dermoscopy: Dermoscopic findings of pigmented lesions of the mucosae.* Barcelona: Cage Editors; 2002.
8. Olszewska M, Banka A, Gorska R et al. Dermoscopy of pigmented oral lesions. *J Dermatol Case Rep.* 2008;2(3):43-48.
9. Lin J, Koga H, Takata M et al. Dermoscopy of pigmented lesions on mucocutaneous junction and mucous membrane. *Br J Dermatol.* 2009;161(6):1255-1261.
10. Blum A, Simionescu O, Argenziano R et al. Dermoscopy of pigmented lesions of the mucosa and the mucocutaneous junction: results of a multicenter study by the International Dermoscopy Society (IDS). *Arch Dermatol.* 2011;147(10):1181-1187.

11. Debarbieux S, Perrot JL, Erfan N et al. Reflectance confocal microscopy of mucosal pigmented macules: a review of 56 cases including 10 macular melanoma. *Br J Dermatol*. 2014;170(6):1276-1284.
12. Cinotti E, Couzan C, Perrot JL, et al. In vivo confocal microscopic substrate of grey colour in melanosis. *J Eur Acad Dermatol Venereol*. 2015;29(12):2458-2462.
13. Maher NG, Solinas A, Scolyer RA et al. In vivo reflectance confocal microscopy for evaluating melanoma of the lip and its differential diagnoses. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2017;123(1):84-94.
14. Erfan N, Hofman V, Desruelles F et al. Labial melanotic macule: a potential pitfall on reflectance confocal microscopy. *Dermatology*. 2012; 224(3):209-211.
15. Laimer M, Arzberger E, Kirchner CA et al. Noninvasive RCM for Differentiation of Melanotic Macules From Melanocytic Lesions-Blinded Evaluation of a Series of 42 Pigmented Macules. *Dermatol Surg*. 2017;43(7):911-919.
16. Uribe P, Collgros H, Scolyer RA, et al. In Vivo Reflectance Confocal Microscopy for the Diagnosis of Melanoma and Melanotic Macules of the Lip. *JAMA Dermatol*. 2017;153(9):882-891.
17. Stolz W, Schiffner R, Burgdorf WH. Dermatoscopy for facial pigmented skin lesions. *Clin Dermatol*. 2002;20(3):276-278.
18. Pralong P, Bathelier E, Dalle S et al. Dermoscopy of lentigo maligna melanoma: report of 125 cases. *Br J Dermatol*. 2012;167(2):280-287.
19. Guitera P, Menzies SW, Longo C et al. In vivo confocal microscopy for diagnosis of melanoma and basal cell carcinoma using a two-step method: analysis of 710 consecutive clinically equivocal cases. *J Invest Dermatol*. 2012;132(10):2386-2394.

- 448 20. Patterson JW, eds. Weedon's skin pathology: Lentigines, nevi, and melanomas. London: Churchill
449 Livingstone Elsevier; 2016.
450
- 451 21. Calonje E, Brenn T, Lazar AJ, Billings SD, eds. McKee's pathology of the skin with clinical
452 correlations: Diseases of the oral mucosa. Amsterdam: Elsevier; 2019.
453
- 454 22. Contaldo M, Agozzino M, Moscarella E et al. In vivo characterization of healthy oral mucosa by
455 reflectance confocal microscopy: a translational research for optical biopsy. Ultrastruct Pathol.
456 2013;37(2):151-158.
457
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Abbreviations

bRCM: blinded reflectance confocal microscopy

ECJ: epithelial-chorion junction

FN: false negative

FP: falso positive

LM: lentigo maligna

MM: malignant melanoma

PLM: pigmented labial macule

RCM: reflectance confocal microscopy

Figure legends:

Figure 1

In situ malignant melanoma, lentigo maligna type (true positive of the RCM Lip Score). (a) Asymmetric pigmented labial macule of 35x10mm 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. (c) RCM image (0.5x0.5 mm). Detail of round pagetoid cells. (d) RCM image (0.5x0.5 mm). Continuous (lentiginous) proliferation of atypical enlarged bright cells in the papilla and sheets of atypical cells at the epithelial-chorion junction. (e)&(f) RCM image (0.5x0.5 mm). Non-edged papilla 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-eosin (H&E), cutaneous component, original magnification x200. (h) Atypical proliferation of melanocytes in the epithelium. Hematoxylin-eosin (H&E), mucosal component, original magnification x200. (i) SOX10 staining, in the mucosal component, labeling the nucleus of melanocytes.

Figure 2

Lentigo maligna (false negative of the RCM Lip Score in the mucosal evaluation). (a) Asymmetric pigmented labial macule of 30x21mm affecting predominantly the cutaneous part of the inferior lip in a man in his 70s. (b) Dermoscopic view: 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. (c) RCM image (0.75x0.75 mm) on the cutaneous part of the lesion. Detail of round pagetoid cells. (d) RCM image (0.75x0.75 mm) on the cutaneous part of the lesion. Detail of a dendritic pagetoid cell. (e) RCM image (0.75x0.75 mm) at the dermal-epidermal junction shows > 3 atypical dendritic cells. (f) RCM image (0.75x0.75 mm). Follicular localization of atypical dendritic cells. (g) RCM image (0.75x0.75 mm). More than 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-eosin (H&E), original magnification x200. Inset: abundant Langerhans cells associated in the epidermis. CD1a stain. (i) Melan-A staining, original magnification x200, labeling the atypical proliferation of melanocytes

(cytoplasm) in the epidermis and adnexa.

Figure 3

Melanotic macule (false positive of the RCM Lip Score). (a) Symmetric pigmented labial macule of 10x7mm on the inferior lip in a woman in her 80s. Dermoscopic view of the medial lesion. It shows light and dark brown lines and grayish structureless zones on the center. Inset: clinical view. (b) RCM image (0.75x0.75 mm). Detail of pagetoid dendritic cells in higher layers of the epithelium. (c) RCM image (0.75x0.75 mm) at the epithelial-chorion junction shows >3 atypical dendritic cells. (d) RCM image (0.75x0.75 mm). Detail of nonhomogeneously distributed papillae around the lesion and presence of 2-3 dendritic bright cells per papillae. (e) Biopsy specimen revealed a melanotic macule. Haematoxylin-eosin, original magnification $\times 200$. 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.

Table 1 Frequencies of dermoscopic and reflectance confocal microscopy parameters

<i>Features</i>	Malignant lesions (n=5)	Benign lesions (n=46)	P value for Malignant vs Benign lesions
Clinical features			
Mean age (SD)	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			
<i>Number of colors</i>			
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
<i>Colors</i>			
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
<i>Number of dermoscopic patterns</i>			
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
<i>Dermoscopic structures</i>			
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
RCM features			
<i>Suprabasal epithelia</i>			
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
<i>Epithelial-chorion junction</i>			
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%	78.3% (36/46)	.098
Nonhomogeneously distributed papillae	80%	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
Nonvisible papillae	0% (0/5)	8.7% (4/46)	>.99
Absence of a hyperreflective 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
Number of basal dendritic cells (1x1mm ²)	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 (0.5x0.5mm ²)	40% (2/5)	34.8% (16/46)	>.99
Number of atypical cells in 1x1mm ² , (SD) [#]	25.2 (26.3)	1.94 (2.8)	.119
Maximum number of dendritic cells per papillae	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	80% (4/5)	39.1% (18/46)	.152

relationship with the papillae			
Network of dendritic cells and dendritic processes	20% (1/5)	21.7% (10/46)	>.99
<i>Chorion</i>			
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 (-2-5)	

NI: not indicated; * Defined as: numerous cells irregular in size, shape, and reflectivity, round to oval or stellate, occasionally with branching dendriticlike structures, and/or distributed throughout the lesion.²²

[#]Atypical cells are defined as cells with irregular size, shape and reflectivity.

Table 2. Specific diagnosis, sensitivity and specificity of each technique

Technique	Specific diagnosis %	Specific diagnosis (benign/malignant) %	S (benign/malignant) %	Sp (benign/malignant) %
Cx-Dx	53.2	89.4	80	90.5
bRCM	47.1	94.1	60	97.8
RCM	62.7	100	100	100

S: sensitivity for melanoma diagnosis; Sp: Specificity for melanoma diagnosis; Cx-Dx: clinical-dermoscopic evaluation; bRCM: reflectance confocal microscopy evaluation according to RCM Lip Score blinded to clinical and dermoscopic images; RCM: reflectance confocal microscopy evaluation aided by clinical-dermoscopic images.

Supplementary material

Methods:

All patients gave written informed consent to be included in the study, which was approved by the Ethics Committee (2014/6000/I and X11-0090&HREC/11/RPAH/123). Clinical and dermoscopic images of all lesions were obtained using a digital camera (Canon G10, Canon Inc) attached to a contact polarized light dermoscopy device (DermLite Foto, 3Gen) in Barcelona and with Heine-20 (Heine Dermatology) in Sydney. RCM examination was performed in all lesions (VivaScope 3000; Caliber I.D. in 39 cases and VivaScope 1500; Caliber I.D. in 12 cases). Confocal images were recorded at three levels of the lip mucosa (epithelium, epithelial-chorion junction (ECJ) and chorion) and multiple vertical sequential images (VivaStack; Caliber I.D.) in the more representative areas.

eFigure1: Melanotic macule (true negative of the RCM Lip Score). (a) Asymmetric pigmented labial macule of 25x15mm on the inferior lip in a woman in her 70s. Dermoscopically it exhibits lines (parallel and reticle), dots and circles of light brown and grayish structureless areas. Inset: clinical view. (b) Reflectance confocal microscopy (RCM) mosaic (1.5x1.5 mm) at the epithelial-chorion junction shows homogeneously distributed dermal papillae, absence of a hyperreflective basal layer, presence of 2-3 dendritic bright cells per papillae and dendritic cells in the interpapillar space. (c) RCM image (0.5x0.5 mm). Detail of dendritic cells at the junction. (d) Biopsy specimen revealed hyperpigmentation at the tip of the rete ridges and melanophages in the chorion, suggestive of melanotic macule. Haematoxylin-eosin, original magnification x100. Inset: detail of melanophages in the chorion (H&E, original magnification x200). (e) Scarce melanocytes (red) in the basal layer of the epithelium. Melan-A stain (red) combined with Ki-67 stain (brown). (f) Detail of dendritic Langerhans cells near the papillae and peripapillae. CD1a stain, transversal section.