Dermatology Practical & Conceptual

Confocal Assessment of Pigmented-Mucosal Lesions: A Monocentric, Retrospective Evaluation of Lip and Genital Area

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ABSTRACT

Introduction: Pigmentation of lip and/or genitalia is mainly due to the development of benign melanotic macules, with a less occurrence of melanocytic and other non-melanocytic lesions. Mucosal melanoma has worse prognosis compared with cutaneous counterpart, hence identification of atypical features for an early diagnosis is crucial.

Objectives: The aim of this study was to report further data of confocal features characterizing pigmented mucosal lesions of genital area and of the lips and test the diagnostic role of the reflectance confocal microscopy (RCM)lip score.

Methods: Clinical, dermoscopic and RCM images of histologically proven pigmented lesions, involving the genital area (vulva or glans penis) and lip, were retrospectively reviewed. RCM images were evaluated for malignant criteria, and statistical analysis was conducted for categorical variables.

Results: Seventy pigmented lesions were included in the study and divided into two groups based on the body area location: lip (17) and genital area (53). Architectural disarray (P = 0.002), dendritic (P = 0.031) and roundish cells in epidermis (P < 0.0001), interpapillary dendritic cells (P = 0.039) and junctional atypical cells (P = 0.002) were associated to genital melanoma. Melanoma involving the lip was characterized by roundish cells in epidermis, a criterion found in one labial benign lesion, only (P = 0.005). Main limitations of the study are the inclusion of low melanomas and the presence of epidermal dendritic cells in melanosis and melanoma, as a confusing factor in imaging.

Conclusions: Dermatologists should consider confocal microscopy as an adjunctive tool to dermoscopy in the differential diagnosis of pigmented mucosal lesions, especially in presence of clinical and dermoscopic findings suspicious for malignancy.

Introduction

Pigmentation of lip and/or genitalia is mainly due to the development of benign melanotic macules (also called melanoses or mucosal pigmented macules), with a less occurrence of melanocytic lesions (naevi and melanoma) and other non-melanocytic lesions (non-melanoma skin cancers, inflammatory conditions, infective diseases, or foreign-body pigmentations) [1,2]. Mucosal melanoma has worse prognosis compared with cutaneous melanoma and lacks effective treatment options, hence identification of atypical features for an early diagnosis is crucial. Dermoscopy has improved diagnostic accuracy of pigmented mucosal lesions (PMLs), indicating multiple colors and structureless areas as strong indicators for malignancy [3,4], supported more recently, by reflectance confocal microscopy (RCM) in the identification of features more associated to mucosal melanoma, like atypical pattern of the epithelium, pagetoid cells and disarranged papillae [2,5-9]. Recently, Uribe et al proposed a RCM lip score that can assist in the differential diagnosis of melanotic macules and melanoma of the lip. Given the rarity of mucosal melanoma only 23 cases including genital and lip area, have been investigated using RCM, and incisional biopsy or surgical excision is still recommended for equivocal lesions [4].

Objectives

The aim of this study was to report further data of confocal features characterizing PMLs of genital area and of the lips, and test the diagnostic role of the RCM lip score.

Methods

Clinical and Imaging Data

A consecutive series of histologically proven PMLs, involving the genital area (vulva or glans penis) and lip, collected at Dermatology Unit, Fondazione Policlinico Universitario A. Gemelli-IRCCS, Rome, within 24 months (March 2016-February 2018) were retrospectively reviewed. Demographic characteristics (patient age, gender, body site) were recorded, and clinical, dermoscopic and RCM images were acquired with digital imaging system (Dermaview DUAL, Tre T Medical snc, Cicciano, Italy; VivaScope® 1500, Caliber Inc). RCM mosaics (VivaBlock®, horizontal sections of 8x8 mm) were taken at the level of epidermis, epithelial-chorion junction (ECJ), and upper chorion, with VivaStack® (frames taken at incremental depths from epidermis to superficial chorion) acquired in areas of special interest. Two investigators (V.C., F.P.) jointly reviewed RCM images blinded for histopathological diagnosis, evaluating RCM features based on previously described criteria [1,8,9]. In case of disagreement, a third experienced dermatologist (A.D.S.) was consulted to reach a consensus. Additionally, the RCM lip score proposed by Uribe et al was considered in the evaluation of pigmented lip lesions [9]. The patients have given written informed consent for their case details. All data were de-identified before use.

Statistical Analysis

Statistical analysis was performed using specific software (SAS Analytics software) and a descriptive evaluation for categorical variables, expressed as the absolute number of cases and percentage values, was conducted. Dowling-Degos disease was not included in the analysis due to the presence of peculiar and distinct RCM features. Fisher exact test was used for the comparison of different confocal parameters between malignant and benign lesions and their association with histological diagnosis. P value less than 0.05 was considered as statistically significant.

Results

Seventy PMLs in 68 patients (52 [76.5%] females; mean age 76.5 [13-78] years) were included in the study and divided into two groups based on the body area location: lip

(17 pigmented lip lesions, PLLs) and genital area (53 pigmented genital lesions, PGLs).

Pigmented Genital Lesions

Fifty-three PGLs (38/53, 74% in women) were evaluated: 29 melanoses, 18 melanocytic nevi, 3 seborrheic keratoses (SK), 2 mucosal melanomas, 1 Dowling–Degos disease. Architectural disarray (P = 0.002), presence of dendritic (P = 0.031) and roundish pagetoid cells in the epidermis (P < 0.0001), interpapillary dendritic cells (P = 0.039) and atypical cells at the DEJ (P = 0.002) were associated to genital melanoma (Table 1).

Melanosis occurred on labia minora (15/22, 68%) and labia majora (7/22, 32%) in women while involved glans penis (3/7, 43%), foreskin of the glans (2/7, 29%) or skin (2/7, 29%) in men. Three melanoses had a multifocal distribution, and 2 melanoses were associated to lichen sclerosus. With RCM, melanoses displayed a regular honeycomb pattern with sparse dendritic cells in 21% of cases. Ringed pattern and draped pattern with homogeneous distribution of papillae represented the main features at epidermal-chorion junction (ECJ) (76% and 55%), along with dendritic cells around papillae (41%), mainly exhibiting a fusiform shape (79%). Junctional atypical cells were seen in 14% lesions (14%) (Figure 1). Melanosis was associated to lichen sclerosus in two patients, revealing in imaging non-specific pattern at epidermal-chorion junction and an inflammatory infiltrate with a prominent vascularization.

Mucosal melanoma was localized on labia minora in a 21-year-old girl and on the glans penis in a 36-year-old man (Figures 1 and 2). RCM images showed honeycomb pattern with epidermal disarray and a widespread infiltration of pagetoid roundish and dendritic cells (>10 cells/mm^2). At the ECJ a meshwork and ringed pattern co-existed, with atypical peri- and inter-papillary dendritic cells (>10 cells/mm^2). Junctional atypical cells (5-10 cells/mm^2 and >10 cells/mm^2) were detected in both cases along with melanocytic nests in the chorion.

Melanocytic lesions included 6/18 (33%) compound nevi, 5/18 (28%) intradermal nevi, 4/18 (22%) AGN and 3/18 (17%) dysplastic nevi. Compound nevi were characterized by regular honeycomb pattern and a prevalent ring pattern (66%) at ECJ. Melanocytic nests in superficial chorion were observed in all lesions. Intradermal nevi showed a regular honeycomb pattern in the epidermis with a predominance of nonedged, ringed or meshwork pattern at ECJ (75%), and melanocytic nests in superficial chorion. AGN mostly revealed honeycomb pattern with epidermal disarray and a sparse or widespread pagetoid infiltration in superficial layers (75%). Meshwork pattern represented the prevalent criterion (75%) at ECJ, along with peri- and inter-papillary dendritic cells (100% and 75% respectively) (Figure 2). In dysplastic nevi, RCM revealed a regular honeycomb pattern in more than half-cases (66%) with sparse dendritic and roundish cells in 33% of cases. DEJ pattern was heterogeneous showing the simultaneous presence of draped and meshwork pattern, or nonspecific pattern. Melanocytic nests were seen in the superficial chorion (100%).

RCM of pSK showed epidermal bulbous projections and keratin-filled invaginations with plump bright cells and bright horn cysts. Ring pattern edged-papillae characterized ECJ.

Dowling Degos disease exhibited a honeycomb pattern in epidermis, junctional ring pattern or elongated and thick cord-like structures, corresponding to irregular, filiform epidermal elongation downward into the upper dermis. Keratin cysts were also seen.

Pigmented Labial Lesions

Seventeen pigmented labial lesions (PLLs) (14/17, 82% in women) were included in the study: 8 melanoses, 3 basal cell carcinomas (BCCs), 2 actinic keratoses (AKs), 1 naevus, 1 atypical nevus, 1 mucosal melanoma, 1 pSK. Although a single melanoma involved the lip, this malignant lesion was characterized by the presence of roundish cells in epidermis, a criterion found in one labial benign lesion, only (melanosis) (P = 0.005) (Table 2).

Melanoses mainly occurred on the lower lip and showed disarranged honeycomb pattern (7/8, 88%) with dendritic cells in half cases (4/8, 50%); DEJ was mainly characterized by a draped pattern (5/8, 63%) and ringed pattern (4/8, 50%) with papillae homogeneously distributed (6/8, 75%); dendritic cells had a prevalent peri-papillary distribution (6/8, 75%) (Figure 3); plump bright cells were seen in 5/8 cases (63%) in papillary dermis.

Melanoma was localized on the lower lip and RCM revealed a broadened honeycomb pattern with dendritic and roundish cells (<5 cells/mm2) in epidermis. DEJ was characterized by non-edged draped pattern, peri-papillary and inter-papillary dendritic cells (5-10 cell per mm^2), showing stellate or fusiform shape (Figure 3).

Compound naevus was found on the upper lip and showed on RCM a regular honeycomb pattern in epidermis, non-edged ringed pattern at DEJ and dermal melanocytic nests. Atypical nevus was located on the lower lip and numerous cyto-architectural atypia were seen on RCM: disarranged honeycomb pattern with dendritic cells (>10 per mm^2) in epidermis, nonspecific pattern at DEJ, numerous peri- and inter-papillary dendritic cells (>10 per mm^2). Melanocytic nests and plump bright cells were observed in papillary dermis.

BCCs were clearly detected by the presence of bright tumor islands and peri-tumoral vessels in papillary dermis.

Table 1. Comparison between benign and malignant pigmented genital lesions.

	Benign pigmented lesions[1] (n=50)	Malignant lesions[2] (n=2)	P Value
Sex			
F n (%)	38/50 (76.0)	1/2 (50.0)	0.405
M n (%)	12/50 (24.0)	1/2 (50.0)	
Age (years) [Mean(±SD)]	44.3(17.6)	28.6 (10.3)	0.219
Localization			
Women			
Labia majora	14/50	0/2	>.99
Labia minora	24/50	1/2	>.99
Men			
Glans	7/50	1/ (50.0)	>.99
Foreskin	2/50	0/1	>.99
Penis	2/50	0/1	>.99
Pubis	2/50	0/1	>.99
Architectural Pattern			
Honeycomb n (%)	49/50 (98.0)	2/2 (100.0)	0.959
Cobblestone n (%)	1/50 (2.0)	0/2 (0.0)	
Epidermal Disarray			0.002
No n (%)	43/50 (86.0)	0/2 (0.0)	
Yes n (%)	7/50 (14.0)	2/2(100.0)	
Broadened HP			0.166
No n (%)	43/50 (86.0)	1/2 (50.0)	
Yes n (%)	7/50 (14.0)	1/2 (50.0)	
Presence of dendritic cells in the epidermis n (%)	14/50 (28.0)	2/2 (100.0)	0.031
Density of cells			
<5 cells/mm^2 n (%)	5/14 (35.7)	0/2 (0.0)	0.230
5-10 cells/mm^2 n (%)	4/14 (28.6)	0/2 (0.0)	
>10 cells/mm^2 n (%)	5/14 (35.7)	2/2 (100.0)	
Distribution of cell [tot. obs. (%)]	12/50 (24.0)	2/50 (4.0)	
Localized n (%)	4/12(33.3)	0/0(0.0)	0.014
Sparse n (%)	7/12(58.3)	0/0(0.0)	
Widespread n (%)	1/12(8.3)	2/28(100.0)	
Roundish cells in epidermis	4/50 (8.0)	2/2 (100.0)	<0.0001
DEJ			
DEJ architecture[§]			
Ringed Pattern	32/50 (64.0)	2/2 (100.0)	0.294
Draped Pattern	19/50 (38.0)	1/2(50.0)	0.732
Meshwork Pattern	5/50 (10.0)	1/2(50.0)	0.083
Clod Pattern	3/50 (6.0)	0/2 (0.0)	0.721
Nonspecific Pattern	4/50 (8.0)	1/2(50.0)	0.048
Distribution of papillae			
Homogeneously	36/50 (72.0)	1/2(50.0)	0.501
Nonhomogeneously	14/50 (28.0)	1/2 (50.0)	
Papillae			
Edged papillae	33/50 (66.0)	1/2(50.0)	0.578
Nonedged papillae	15/50 (30.0)	1/2(50.0)	
Mixed	2/50 (4.0)	0/2 (0.0)	
Presence of peripapillary dendritic cells	20/50 (40.0)	2/2(100.0)	0.092
Density of cells			
<5 cells/mm^2	7/20 (35.0)	0/2 (0.0)	0.204
5-10 cells/mm^2	6/20 (30.0)	0/2 (0.0)	
>10 cells/mm^2	7/20 (35.0)	2/2 (100.0)	
Presence of interpapillary dendritic cells	15/50 (30.0)	2/2 (100.0)	0.039
Density of cells			
<5 cells/mm^2	6/15 (40.0)	0/2 (0.0)	0.365
5-10 cells/mm^2	2/15 (13.3)	0/2 (0.0)	
>10 cells/mm^2	7/15 (46.7)	2/2 (100)	
Morphology of dendritic cells[§] **[tot. obs. (%)]**	22/50 (44.0)	2/2 (100.0)	
fusiform	17/22 (77.3)	2/2 (100.0)	0.449
stellate	7/22 (31.8)	1/2 (50.0)	0.602
triangular	7/22 (31.8)	1/2 (50.0)	0.602
Presence of atypical cells at DEJ	7/50 (14.0)	2/2 (100.0)	0.002
Density of cells			
<5 cells/mm^2	2/7 (28.6)	0/5(0,0)	0.669
5-10 cells/mm^2	3/7 (42.99	½(50.0)	
>10 cells/mm^2	2/7 (28.6)	½(50.0)	
Papillary dermis			
Papillary nests	18/50 (36.0)	2/2(100)	0.068
Tumor Islands	0/50 (0.0)	0/50 (0.0)	
Vessels visible	5/50 (10.0)	0/2 (0.0)	0.638
Plump bright cells	14/50 (28.0)	0/2 (0.0)	0.381

[1] Benign pigmented lesions: melanosis, atypical melanocytic lesion, naevus, pigmented seborrheic keratosis, atypical nevus of genital type
[2] Malignant lesions: melanoma

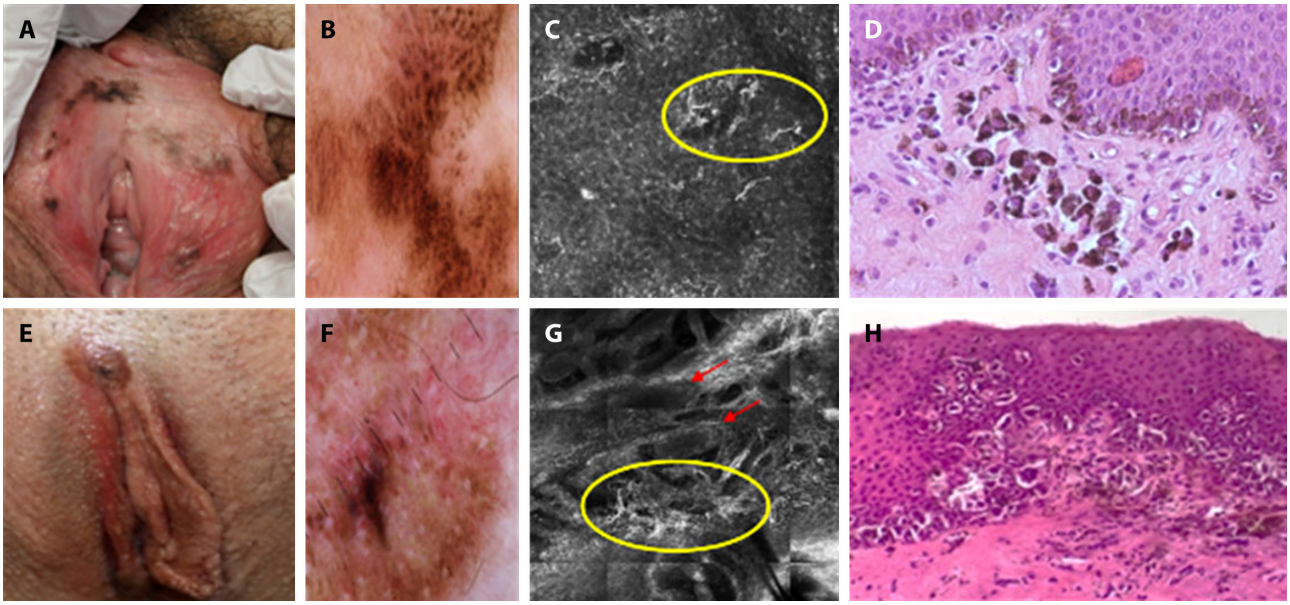

Figure 1. (A-D) Melanosis of a 45-year-old woman and (E-H) invasive melanoma (Breslow tumor thickness, 0,2 mm) of a 30-year-old woman: (A) Multiple pigmented macules in the labia minora, exhibiting with dermoscopy (B) parallel lines, clods and brown color, and (C) with RCM focal dendritic cells in epidermis (yellow circle); (D) Histopathology features of increased basal keratinocyte pigmentation mostly restricted to tips of rete ridges, mild increase in the number of melanocytes (no cytologic atypia and/or nests) at dermo-epidermal junction, melanin pigment incontinence and melanophages in the upper chorion/lamina propria (H&E stain, original magnification × 200); (E) Asymmetric pigmented macule of the skin adjacent to the clitoris (F) showing structureless pattern, circles, and multiple colors with dermoscopy; (G) Confocal revealing junctional non-specific pattern with numerous atypical cells (yellow circle) and melanocytic nests (red arrows); (H) histopathology section exhibiting a radial proliferation of atypical melanocytes at epidermal basal layer and upper lamina propria with focal pagetoid spread (H&E stain, original magnification × 150).

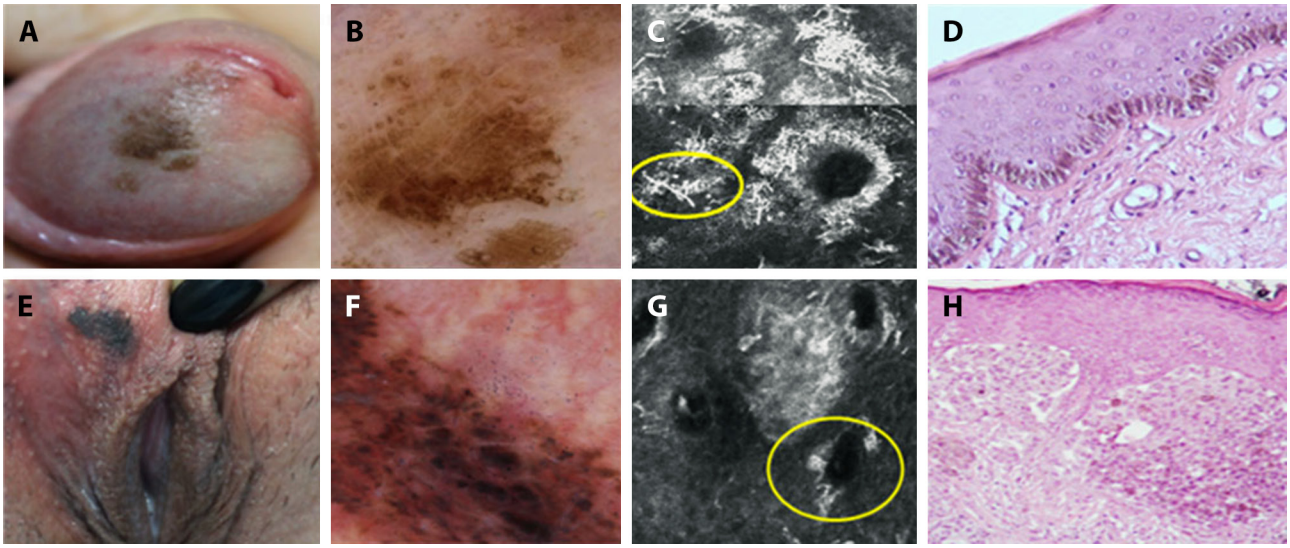

Figure 2. (A-D) In situ melanoma of a 40-years-old man and (E-H) atypical melanocytic nevus of genital type of a 15-year-old woman: (A) brown irregular macule on the glans penis (B) exhibiting reticular lines and circles with dermoscopy; (C) Confocal unveils inter- and peri-papillary atypia with sheets of atypical cells at the epithelial-chorion junction (yellow arrow); (D) Histopathology showing a radial proliferation of contiguous, mildly atypical melanocytes at the basal layer of epidermis, with focal pagetoid spread in the supra-basal layer (H&E stain, original magnification × 200); (E) Dark pigmented irregular macule on labia majora displaying (F) globules and clods, reticular lines and brown color on dermoscopy; (G) confocal image of pagetoid spreading with dendritic and roundish cells in epidermis (yellow circles), with (H) histology showing irregularly shaped and sized nests, large and roundish, with bizarre balloon or cannonball appearance (H&E stain, original magnification × 200).

Table 2. Comparison between benign and malignant pigmented labial lesions.

	Benign pigmented lesions[1] (n=16)	Malignant lesions[2] (n=1)	P Value
Sex			
F	13/16 (81.3)	1/1 (100.0)	0.633
M	3/16(18.7)	- (0.0)	
Mean Age (years)	50.0 (19.0)	42	
Localization			
Upper lip	5/16 (31.3)	0/1 (0.0)	0.801
Lower lip	11/16 (68.7)	1/1 (100.0)	
Epidermis			
Architectural pattern			
Honeycomb	15/16 (93.7)	1/1 (100.0)	0.797
Cobblestone	1/16 (6.3)	0/1 (0.0)	
Epidermal Disarray	6/16 (37.5)	0/1 (0.0)	0.446
Broadened HP	8/16 (50.0)	1/1 (100.0)	0.331
Presence of Dendritic cells in the epidermis	6/16 (37.5)	1/1 (100.0)	0.218
Roundish cells in epidermis	1/16 (6.3)	1/1 (100.0)	**0.005**
DEJ			
DEJ architecture [tot. Obs(%)]	15/16 (93.7)	1/1 (100.0)	
Ringed Pattern	6/15(40.0)	0/1 (0.0)	0.309
Draped Pattern	4/15 (26.7)	1/1(100.0)	
Nonspecific Pattern	5/15 (33.3)	0/1 (0.0)	
Distribution of papillae			
Homogeneously	10/16(62.5)	1/1 (100.0)	0.446
Nonhomogeneously	6/16 (37.5)	0/1 (0.0)	
Papillae[§] [tot. Obs(%)]	15/16 (93.7)	1/1 (100.0)	
Edged papillae	4/15 (26.7)	0/1 (0.0)	0.587
Nonedged papillae	7/15 (46.6)	1/1 (100.0)	
Nonvisible papillae	4/15 (26.7)	0/1 (0.0)	
Presence of Peripapillary dendritic cells	9/16 (56.2)	1/1 (100.0)	0.388
Presence of Interpapillary dendritic cells	5/16 (31.2)	1/1 (100.0)	0.163
Morphology of dendritic cells[§] [tot. Obs (%)]	9/16 (56.2)	1/1 (100.0)	
fusiform	8/9	1/1 (100.0)	0.998
stellate	3/9	1/1(100.0)	
triangular	3/9	0/1(0.0)	
Presence of atypical cells at DEJ	2/16(12.5)	0/1 (0.0)	0.707
Papillary dermis			
Papillary nests	2/16 (12.5)	0/1 (0.0)	0.707
Tumor Islands	3/16 (18.7)	0/1 (0.0)	0.633
Vessels visible	6/16 (37.5)	0/1 (0.0)	0.446
Plump bright cells	10/16 (62.5)	0/1 (0.0)	0.218

[1] Benign pigmented lesions: melanosis, naevi, atypical melanocytic naevus, pigmented seborrheic keratosis
[2] Malignant lesions: melanoma

AKs disclosed disarranged honeycomb pattern and dendritic cells in epidermis; nonspecific pattern was observed at DEJ with peri- and inter-papillary dendritic cells.

Images of SK revealed a regular honeycomb pattern with bulbous projections and invaginations in epidermis, plump bright cells at DEJ and in the upper dermis.

Conclusions

Diagnosis of PMLs may be challenging with clinical/dermoscopic examination alone [4,5]. Melanosis is the most frequent cause of mucosal pigmentation, although a skin cancer, an inflammatory condition, foreign-body pigmentation and pigmented cicatricial scar may rarely occur in clinical setting [1,2]. In this study 70 PMLs were assessed based on a large series of RCM criteria. Epidermal disarray, pagetoid cells, junctional atypia was statistically associated to a diagnosis of mucosal melanoma, supporting results of a recent review that identified pagetoid large cells, high density of basal dendritic cells and loss of chorion normal architecture as the major features of mucosal melanoma [10].

In our series, dendritic cells in epidermis were detected in mucosal melanoma and in considerable proportion of melanoses (6/29, 21%), potentially representing a confounding factor and a major problem for differential diagnosis. Lamier et al defined "irregular dendritic type", a subtype of melanoses showing RCM overlapping features with mucosal melanoma: epidermal atypical cells, junctional atypia and focal loss of regular architecture. In presence of epidermal atypia, the density of dendritic cells may represent an important clue, since ≥10 cells/mm^2 are more suggestive of mucosal melanoma [8,10]. Roundish cells at DEJ, seen in 4/29

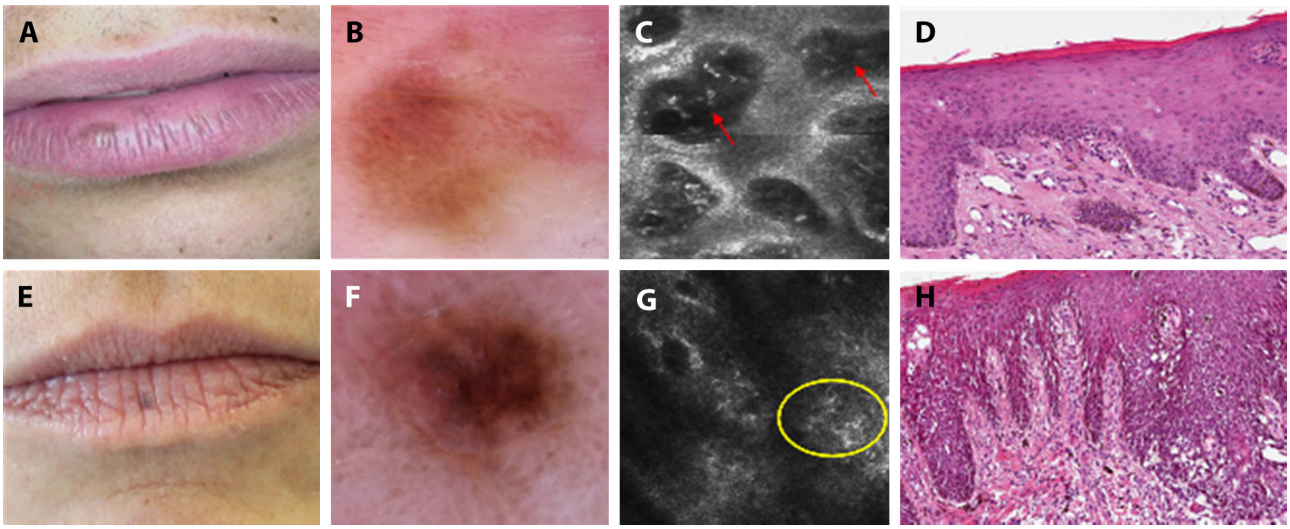

Figure 3. (A-D) Melanosis of a 16-year-old man and invasive melanoma (Breslow tumor thickness, 0,2 mm) of a 42-year-old woman: (A) pigmented macule on the lower lip (B) with structureless pattern, parallel lines and brown color on dermoscopy; (C) Confocal images of the junction displaying draped pattern edged-papillae with papillary small bright cells (red arrows), (D) histologic section showing increased basal keratinocyte pigmentation and a mild increase of non-atypical melanocytes at the dermo-epidermal junction (H&E stain, original magnification × 200); (E) Brownish macule on the lower lip with (F) dermoscopic features of brown structureless areas (G) confocal findings of non-edged papillae, atypical cells at the junction (yellow circle); (H) Histopathology section exhibiting a proliferation of atypical melanocytes at epidermal basal layer and upper lamina propria with focal pagetoid spread (H&E stain, original magnification × 200).

melanoses (14%), are generally also detected in lentiginous pattern of in situ mucosal melanoma, originating from an atypical melanocytic hyperplasia ie the so-called "intraepidermal atypical melanocytic proliferation of uncertain significance (IAMPUS)" [11]. Following a continuous model, in its early phase, melanoma may show minimal cytological atypia without clear architectural disarrangement, mimicking a melanosis. Thus, RCM can be useful in the monitoring of atypical PMLs over time detecting minimal worrisome changing.

AGN represent a subset of benign nevi occurring in young adults that show worrisome clinical features such as dark pigmentation, irregular borders and large size, and suspicious dermoscopic findings, like mixed pattern and multiple colors [4,12]. Herein, AGN were found in young girls (14-18-years-old) exhibiting clinical features of dark brown color, fast growth and large size (>1cm in diameter). RCM was not useful for their correct recognition due to relevant cyto-architectural irregularities, leading therefore to surgical excision. It is noteworthy that diagnosis of AGN is also challenging with histopathology, because of the presence of architectural disorder, nested pattern and pagetoid spreading, with various degree of cellular atypia [12].

Pigmentary changes following inflammatory conditions, like occurring with lichen sclerosus, may be a cause of concern often requiring histopathological examination [13,14]. Confocal displayed classical features of melanosis in our two cases, leading to a conservative approach.

Results from PLLs revealed roundish cells in epidermis as the unique criterion significantly associated with melanoma ($P = 0.005$). Calculating the RCM lip score of Uribe et al, it was ≥ 4 in atypical/malignant lesions and lower in benign lesion, providing further evidence of its utility, as already suggested by Gomez-Martin et al, evaluating 51 PLLs, of which 5 mucosal melanomas [1]. In our series 2/8 melanoses (25%) were considered false positives obtaining a LIP score ≥ 4, due to disarray and dendritic cells in epidermis and junctional atypical cells. Such discrepance could be related to the presence of epidermal inflammation, as confirmed by histopathology revealing numerous Langerhans cells in suprabasal layers [1,9].

Diagnosis of benign (SK, AK) and malignant (BCC) keratinocyte skin lesions was simplified by the recognition of common confocal criteria [15-17].

Main limitation of the study is the inclusion of only 3 cases of mucosal melanoma, of which 1 melanoma of the lip, probably related to rarity of this entity in clinical setting. Additionally, the detection in RCM of dendritic bright cells in the epithelium of melanosis and melanoma, may represent a confusing factor for diagnosis, although density, shape and location of this cells may add important clues.

Dermatologists should consider confocal microscopy as an adjunctive technique to dermoscopy, for the differential diagnosis of PMLs showing clinical and dermoscopic findings suspicious for malignancy, and for long-term surveillance of atypical melanocytic lesions. Anyhow, PMLs

showing overlapping features with melanoma should undergo to incisional biopsy or surgical excision for not to miss an early diagnosis of malignancy.

References

1. Gómez-Martín I, Collgros H, Ferguson PM, et al. Diagnostic accuracy of pigmented labial macules by in vivo reflectance confocal microscopy and correlation among techniques. *J Am Acad Dermatol*. 2021;85(5):1151-1160. DOI: 10.1016/j.jaad.2020.02.067. PMID: 32147389.
2. Debarbieux S, Perrot JL, Erfan N, et al. Reflectance confocal microscopy of mucosal pigmented macules: a review of 56 cases including 10 macular melanomas. *Br J Dermatol*. 2014;170(6):1276-84. DOI: 10.1111/bjd.12803. PMID: 24359328.
3. Lin J, Koga H, Takata M Saida T. Dermoscopy of pigmented lesions on mucocutaneous junction and mucous membrane. *Br J Dermatol*. 2009;161(6):1255-1261. DOI: 10.1111/j.1365-2133.2009.09251.x. PMID: 19673880.
4. 4 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. DOI: 10.1001/archdermatol.2011.155. PMID:21680757.
5. 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. DOI: 10.1111/jdv.13394. PMID: 26403597.
6. Maher NG, Solinas A, Scolyer RA, Guitera P. 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. DOI: 10.1016/j.oooo.2016.08.011. PMID: 27720652.
7. Erfan N, Hofman V, Desruelles F, et al. Labial melanotic macule: a potential pitfall on reflectance confocal microscopy. *Dermatology*. 2012;224(3):209-211. DOI: 10.1159/000336775. PMID: 22414589.
8. Laimer M, Arzberger E, Kirchner CA, Prodinger C, Hofmann-Wellenhof R, Ahlgrimm-Siess V. 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. DOI: 10.1097/DSS.0000000000001110. PMID: 28430732.
9. Uribe P, Collgros H, Scolyer RA, Menzies SW, Guitera P. In vivo reflectance confocal microscopy for the diagnosis of melanoma and melanotic macules of the lip. *JAMA Dermatol*. 2017;153(9):882-891. DOI: 10.1001/jamadermatol.2017.0504. PMID: 28467525. PMCID: PMC5710424.
10. De Pascalis A, Perrot JL, Tognetti L, Rubegni P, Cinotti E. Review of Dermoscopy and Reflectance Confocal Microscopy Features of the Mucosal Melanoma. *Diagnostics (Basel)*. 2021;11(1):91. DOI: 10.3390/diagnostics11010091. PMID: 33429900. PMCID: PMC7827612.
11. Elder DE, Bastian BC, Cree IA, Massi D, Scolyer RA. The 2018 World Health Organization Classification of Cutaneous, Mucosal, and Uveal Melanoma: Detailed Analysis of 9 Distinct Subtypes Defined by Their Evolutionary Pathway. *Arch Pathol Lab Med*. 2020;144(4):500-522. DOI: 10.5858/arpa.2019-0561-RA. PMID: 32057276.
12. Ribé A. Melanocytic lesions of the genital area with attention given to atypical genital nevi. *J Cutan Pathol*. 2008;35 Suppl 2:24-27. DOI: 10.1111/j.1600-0560.2008.01150.x. PMID: 18976416.
13. Sollena P, Caldarola G, Di Stefani A, Massi G, Peris K. Lichen sclerosus of the glans simulating melanoma. *J Am Acad Dermatol*. 2017;76(2S1):S49-S51. DOI: 10.1016/j.jaad.2016.07.015. PMID: 28087029.
14. Vaccari S, La Placa M, Barisani A, et al. Penile Exogenous Pigmentation Mimicking Melanoma. *Dermatol Pract Concept*. 2022;12(1):e2022014. DOI: 10.5826/dpc.1201a14. PMID: 35223159. PMCID: PMC8825230.
15. Scharf C, Argenziano G, Brancaccio G, Licata G, Ronchi A, Moscarella E. Melanocytic or Not? Dermoscopy and Reflectance Confocal Microscopy for Lesions Difficult to Diagnose: A Cross-Sectional Diagnostic Accuracy Study. *Dermatol Pract Concept*. 2021;11(4):e2021127. DOI: 10.5826/dpc.1104a127. PMID: 34631270. PMCID: PMC8480465.
16. Cinotti E, Tonini G, Perrot JL, Habougit C, Luisi S, Rubegni P. Dermoscopic and reflectance confocal microscopy features of two cases of vulvar basal cell carcinoma. *Dermatol Pract Concept*. 2018;8(1):68-71. DOI: 10.5826/dpc.0801a17. PMID: 29445582. PMCID: PMC5808379.
17. Navarrete-Dechent C, Uribe P, Rabinovitz H, Abarzua-Araya A, Kittler H. Basal Cell Carcinomas Presenting as Flat Pigmented Macules on the Face Mimicking Lentigo Maligna on Dermoscopy: A Case Series. *Dermatol Pract Concept*. 2023;13(1):e2023038. DOI: 10.5826/dpc.1301a38. PMID: 36892346. PMCID: PMC9946124.