Dermatology Practical & Conceptual

Dermoscopy and Reflectance Confocal Microscopy to Estimate Breslow Index and Mitotic Rate in Primary Melanoma

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ABSTRACT

Introduction: Non-invasive imaging techniques offer the possibility to optimize the first approach to melanoma. Reflectance Confocal Microscopy (RCM) has a promising role in predicting the main prognostic events in the dermo-epidermal and papillary dermis.

Objectives: To identify pre-surgical criteria that can predict the main prognostic features of melanoma.

Methods: A retrospective cohort-study evaluated dermoscopic, confocal and histopathological characteristics of consecutively diagnosed sporadic melanomas. RCM-melanoma patterns classified into 1) dendritic-cell, 2) round-cell, 3) dermal nest and 4) combined type. Acral, facial and mucosal locations were excluded.

Results: Ninety-two primary melanomas were included: 44 males and 48 females (mean age 60.4 years, standard deviation [SD] 16.2) with a mean Breslow of 1.43 mm (SD 1.6). The most frequent

dermoscopic presentation was the multicomponent pattern, the predominant confocal pattern was dendritic-cell type (44.6%). The presence of pigmented network on dermoscopy was related to lower Breslow and mitotic rates (both P = 0.002); in contrast to the presence of visible vessels, which was related to higher Breslow and mitotic indexes (both P = 0.001). Confocal observation of dermal nests or atypical cells in the papillary dermis was related to a higher mitotic rate (P = 0.006 and P = 0.03, respectively). Similarly, diffuse inflammatory infiltrates visible in the superficial dermis was associated with higher Breslow (P = 0.04) and mitotic index (P = 0.04).

Conclusions: Dermoscopic and RCM in vivo findings on primary melanoma correlate with histopathologic Breslow index, mitotic rate and tumor infiltrating lymphocytes. The architecture and cytology of primary melanoma can be estimated by combining dermoscopy and RCM prior to excision.

Introduction

Currently, the 10-year cause-specific survival rate of patients diagnosed with localized cutaneous melanoma (that is, in the absence of nodal or visceral involvement) varies from 75% to 98% [1]. Promising results from molecular profiling of melanoma will shed some light on the prognostic classification of primary tumors [2]; the revised 8th version of AJCC, however, considers that the main prognostic markers of cutaneous melanoma are still ulceration and Breslow depth index of the primary tumor. Mitotic rate is no longer included in the current AJCC; nevertheless, the Melanoma Expert Panel recognizes mitotic rate as an important prognostic parameter to predict sentinel lymph node status and outcome in N0 stage [3].

The prediction given by the prognostic markers prior to removing the tumor could 1) optimize the first approach to the patient: devising an accurate surgery plan and indications for staging tests, and 2) help to preserve fresh samples for further molecular studies in the areas of interest.

Dermatological non-invasive imaging techniques have opened a new era of in vivo tumor characterization. Dermoscopically, several attempts have been made to predict mitotic and Breslow indexes, and even the sentinel lymph node status, based on the various in vivo morphologic features of primary melanoma [4-6].

Reflectance confocal microscopy (RCM) provides real-time evaluation of skin by generating horizontal optical sections from the epidermis to the papillary dermis with cellular-level resolution. Multiple studies have demonstrated not only the high correlation of dermoscopic and confocal features with histopathology, but also the impact of RCM on melanoma diagnostic accuracy - an ancillary tool to dermoscopy, especially with challenging and amelanotic tumors [7-11]; and in a recent Chochrane systematic review, RCM demonstrated may have a potential role in the assessment of lesions that are difficult to diagnose using visual inspection and dermoscopy alone [12].

Additionally, four different RCM melanoma patterns have been described in order to classify melanomas based on their cytology and architectural morphology: 1) dendritic-cell melanomas, 2) round-cell melanomas, 3) dermal nest melanomas and 4) combined type (that may represent an evolution of the other three types) [13,14].

Objectives

Beyond the morphological classification of primary tumors, the goal of the present study was to identify pre-surgical dermoscopic and confocal features of primary tumors that can be correlated with prognostic markers in localized cutaneous melanoma.

Methods

A retrospective cohort study of consecutively pathologically proven cutaneous melanomas diagnosed in a referral unit was conducted. Inclusion criteria stipulated available tumors with high quality clinical, dermoscopic and confocal images prior to excision, and pathological samples to re-review. Only sporadic cases of melanoma were included. Mucosal, acral and facial locations were excluded. The inclusion period was from February 2011 to February 2015.

Stored high-resolution clinical-dermoscopy images were obtained by Canon G11 and Dermlitephoto® (3 GEN, LLC). RCM images obtained by Vivascope 1500 (Lucid Inc.) included a minimum of three mosaics on a horizontal plane ("VivaBlock" modality) covering a maximum area of 7 mm^2, acquired in the spinous-granular layer, the dermal-epidermal junction and the upper dermis respectively. Furthermore, several confocal sections at 500 x 500 microns with vertical montage images (stack images) from stratum corneum to papillary dermis (maximum depth achieved, 250 µm) were acquired in the areas of interest.

Based on the literature, twenty clinical-dermoscopic criteria, including a quantitative Total Dermoscopy Score (TDS) [15], the 7-checkpoint list [16] and 22 confocal features, were established. Confocal characterization of primary melanomas according to the Pellacani et al classification and the confocal Barcelona algorithm, were also assessed [9,13].

In addition, confocal features were evaluated according to the percentage of the lesion surface that each presented: 25%, 50%, 75% or 100% of the lesion. For the histological review, 12 histological routine features were evaluated (Breslow, ulceration, radial or vertical growth phase, mitotic index per mm^2, and also 4 characteristics described by Vios et al [17]**:** nesting or scatter growth, pigmentation and cell shape (summarized in Tables 2 and 3). The Hospital Clínic Ethics Committee approved the protocol and all procedures followed the principles of the Declaration of Helsinki.

Statistical Analysis

SPSS Statics for Windows, version 22.0 (IBM Corp. Released 2013) was used to assess: descriptive values of all qualitative variables by Pearson's Chi Square test; quantitative (age, size of lesions, dermoscopic and confocal scores, and Breslow index) and semi-quantitative scales (such as mitosis, regression, nesting, pagetoid extension) by Student t-test. Comparisons between categorical variables and the correlation between them were analyzed using Pearson's chi-square and Fisher exact test when any cell had an expected count of less than 5. For quantitative continuous variables, Student t-test was used for comparison between two groups and ANOVA where more than two were compared. Person coefficient was used to study the correlation between variables and Spearman correlation coefficient if nonparametric applicability conditions were not reached. The level of statistical significance was set at bilateral 5%. The linear regression model was applied to identify independent markers among quantitative variables.

Results

From the 132 patients who were initially included, 40 were excluded due to poor quality of images or the unavailability of histopathologic samples for reevaluation. All 92 patients were finally included with demographics and clinical staging collected from their medical records.

Clinical-dermoscopic Attributes

In total, 92 patients, 44 males (47.8%) and 48 females (52.2%) with a mean age of 60.4 years (range 15-86) were studied. Most of the tumors were located on the trunk (57.6%), the remaining on the limbs. The most common overall dermoscopic patterns were multicomponent in 51 (55.4%) and reticular in 27 (29.3%). Dermoscopic erosions/ulceration was observed in 18 tumors (19.6%).

Reflectance Confocal Microscopy Attributes

Table 1 summarizes the dermoscopic and confocal features evaluated. According to the melanoma RCM classification [13],

Table 1. Dermoscopic and reflectance confocal features

Dermoscopic Features	Primary Melanomas (N= 92)
Global pattern	
Reticular	27 (29.3%)
Multicomponent	51 (55.4%)
Unspecific	12 (13.0%)
Globular	1 (1.1%)
Homogeneous	1 (1.1%)
Pigmented Network	
Absent	20 (21.7%)
Typical	6 (6.5%)
Atypical	66 (71.7%)
Globules	
Absent	27 (29.3%)
Typical	7 (7.6%)
Atypical	58 (63.0%)
Streaks	
Absent	35 (38.0%)
Typical	16 (17.4%)
Atypical	41 (44.6%)
Structureless areas	
Absent	70 (76%)
Present	22 (24%)
Blue-white veil	
Absent	61 (66.3%)
Present	31 (33.7%)
Regression	
Absent	49 (53.3%)
Present	43 (46.7%)
Milky-red areas	
Absent	60 (65.2%)
Present	32 (34.8%)
Shiny-white streaks	
Absent	37 (40.2%)
Present	55 (59.2%)
Rosettes	
Absent	75 (81.5%)
Present	17 (18.5%)
Vessels	
Absent	48 (52.2%)
Present	44 (47.8%)
Dotted	25 (27.2%)
Hairpin	8 (8.70%)
Polymorphic	27 (29.3%)
Ulceration	

Table 1 (*Continued*)

Table 1. Dermoscopic and reflectance confocal features. (*Continued*)

Dermoscopic Features	Primary Melanomas (N = 92)
Absent	74 (80.4%)
Present	18 (19.6%)
Atypical Blotches	
Absent	63 (68.5%)
Present	29 (31.5%)
Negative network	
Absent	74 (80.4%)
Present	18 (19.6%)
Confocal Characteristics	**Primary Melanomas (N = 92)**
Epidermal Cobblestone	
Absent	38 (41.3%)
Typical	21 (22.8%)
Atypical	33 (35.9%)
Epidermal Honeycomb	
Absent	7 (7.6%)
Typical	39 (24.4%)
Atypical	46 (50.0%)
Epidermal Disarranged	
Absent	34 (37.0%)
Present	58 (63.0%)
Large pagetoid round cells	
Absent	14 (15.3%)
Present	78 (84.7%)
Large pagetoid dendritic cells	
Absent	31 (33.7%)
Present	61 (66.3%)
Atypical pagetoid pleomorphic cells	
Absent	84 (91.3%)
Present	8 (10.2%)
Pagetoid star-shaped cells	
Absent	90 (97.8%)
Present	2 (2.2%)
Mainly edged papilla	
Absent	53 (57.6%)
Present	39 (42.4%)
Typical cells at the basal	
Absent	77 (83.7%)
Present	15 (16.3%)
Atypical Basal Round cells	
Absent	20 (21.7%)
Present	72 (78.3%)
Atypical Basal Dendritic cells	
Absent	33 (35.9%)
Present	59 (64.1%)
Basal atypia	
Absent	2 (2.2%)
Moderate	40 (43.5%)
Marked	50 (54.3%)
Nucleated dermal cells	
Absent	56 (60.9%)
Present	36 (39.1%)
Dermal Nests	
Absent	39 (42.4%)
Present:	53 (57.6%)
Dense	54 (58.7%)
Sparse cell	1 (1.1%)
Cerebriform	5 (5.4%)
Inflammatory cells in dermis	
Plump cells	40 (43.5%)
Bright particles	88 (95.7%)
Collagen bundles	
Visible	59 (64.1%)
Non visible	33 (35.9%)
Dermal vessels	
Absent	69 (75.0%)
Present	23 (25.0%)
Horizontal	11 (12.0%)
Coiled	2 (2.2%)
Thin / Thick	3 (3.3%) / 20 (21.7%)

subtypes fell into 4 groups: the predominant confocal subtype was the so-called dendritic type (41, 44.6%), the round cells type (40, 43.5%), followed by the combined (9, 9.8%). One case was considered dermal nest type (1.1%) and one case was non-classifiable (1.1%).

The Barcelona algorithm score was equal to or higher than 0 in 84 out of 92 cases (91.3%), which is highly suggestive of melanoma; the remaining 8 (8.7%) achieved a score of -1, which means it could be melanoma [9].

Histological Characterization

The most common histological subtype observed was superficial spreading melanoma (SSMM) in 52 (56.6%) of the cases, followed by in situ melanomas in 28 lesions (30.4%), lentigo maligna melanoma (LMM) in 6 (6.5%), nodular (NM) in 5 (5.4%) and one spitzoid melanoma (1.1%). Only 6.5% of melanomas presented histological ulceration. According to tumor infiltrating lymphocytes (TILs), 58 cases (63%) were

considered non-brisk, while 28 (30.4%) were considered brisk; in 6 (6.5%), TILs were absent. The predominant cytology was epithelioid (ovoid) in 49 (53.3%) cases. Growth pattern was classified into marked scattered or pagetoid in 25 (27.2%) cases, moderate in 22 (23.9%) and mild in 38 (41.3%), while nesting pattern was considered marked in 28 (30.4%), moderate in 11 (12%) and mild in 30 (32.6%). Cytoplasmic pigmentation was absent in 11 cases (12%), faint in 26 (28.3%), moderate in 35 (38%), high in 13 (14.1%), and very high in 7 (7.6%).

Some degree of solar elastosis was present in 70 patients (80.4%). Only 2 patients presented vascular invasion and 1 neural invasion. Regression was classified as marked (> 50% of surface) in 20 (21.7%), partial (< 50%) in 27 (29.3%), focal in 20 (21.7%) and absent in 25 (27.2%) cases. Among the invasive cases (N = 64) the mean Breslow index was 1.43 mm (SD 1.6). The mean mitotic rate was 1.25/mm^2 (SD 2.0): in 29 invasive cases (45.3%) less than 1 mitosis/mm2, and 10 cases (15.6%) between 1 and 3 mitosis/mm^2. The remaining 12 cases (18.7%) showed a high mitotic index (4 or more mitosis/mm^2). Table 2 summarizes the histopathology in detail.

Association of in Vivo Findings with Histological Prognostic Markers (Table 3)

Dermoscopic Correlations

As regards the global dermoscopic pattern, a multicomponent pattern was associated with higher nesting growth on histology (P = 0.05) while a reticular pattern was protective against nesting on histology (P = 0.001).

The presence of a pigment network was related to a lower Breslow index and lower mitotic rate (both P = 0.002), while the presence of blotches, structureless pigmentation, visible vessels and blue white veil were related to a higher Breslow index and mitotic rate (P = 0.05, p < 0.01, P < 0.01 and P < 0.01 respectively).

Multivariate analysis demonstrated that a dermoscopic pigment network was related to a lower Breslow index and a lower mitotic rate (both P = 0.002), while the presence of atypical blotches was related to a higher Breslow index and mitotic rate (P = 0.05, P < 0.01).

Confocal Correlations

The presence of typical epidermal cobblestone and honeycomb patterns was more likely to be associated with in-situ melanomas (51.7% and 53.6% of in situ cases, P = 0.04), and as expected, the presence of mainly edged papillae as well (48.7% of in situ versus 20.1% of invasive cases, P = 0.005).

The observation of atypical epidermal cobblestone was related to lentigo maligna subtype (LMM), seen in 50% of cases, in contrast to 34.8% of non-LMM cases (P = 0.04).

Table 2. Histopathological characterization

Histological features	Primary melanomas (n:92)
Histological Type	
In situ	28 (30.4%)
Superficial spreading	52 (56.5%)
Lentigo maligna	6 (6.5%)
Nodular	5 (5.4%)
Spitzoid	1 (1.1%)
Clark Level	
II	10 (10.9%)
III	22 (23.9%)
IV	30 (32.6%)
Mitotic Rate	
< 1/mm^2	29 (45.3%)
1-3/mm^2	10 (15.6%)
> 4/mm^2	12 (18.7%)
Pagetoid infiltration grade / Scattered cells	
0 none	7 (7.6%)
1 mild	38 (41.3%)
2 moderate	22 (23.9%)
3 marked	25 (27.2%)
Nesting grade	
0 none	23 (25%)
1 mild	30 (32.6%)
2 moderate	11 (12.0)
3 marked	28 (30.4%)
Cytoplasmatic Pigmentation	
Faint	26 (28.3%)
Moderate	35 (38.0%)
High	13 (14.1%)
Very High	7 (7.6%)
Cytomorphology	
Round	29 (31.5%)
Ovoid	49 (53.3%)
Elongated	10 (10.9%)
Spindled	4 (4.3%)
Elastosis	
Low	28 (30.4%)
Moderate	29 (31.5%)
Marked	17 (18.5%)
Ulceration	
Present	6 (6.5%)
TIL s Brisk	
Non-Brisk	58 (63.0%)
Brisk	34 (37.0%)
Type of growth	
Vertical	62 (67.4%)
Radial	30 (32.6%)
Regression	
>50%	20 (21.7%)
<50%	27 (29.3%)
Focal	20 (21.7%)
Vascular Invasion	
Present	2 (2.2%)
Neural Invasion	
Present	1 (1.1%)

Table 3. Dermoscopic and confocal features with prognostic value (P < 0.05).

Higher		Lower	
Breslow:	**Mitotic Rate:**	**Breslow:**	**Mitotic Rate:**
Dermoscopic Criteria:			
Atypical blotches			
Structureless pigmentation			
Visible vessels			
RCM Criteria:			
Bright particles and plump cells in dermis		Edged papillae and typical epidermis[a]	Typical cells in basal layer
	Dermal nests		
	Atypical nucleated cells in dermis		

[a] Existence of typical honeycomb and cobblestone patterns in epidermis, and edged papillae were associated with in situ melanomas.

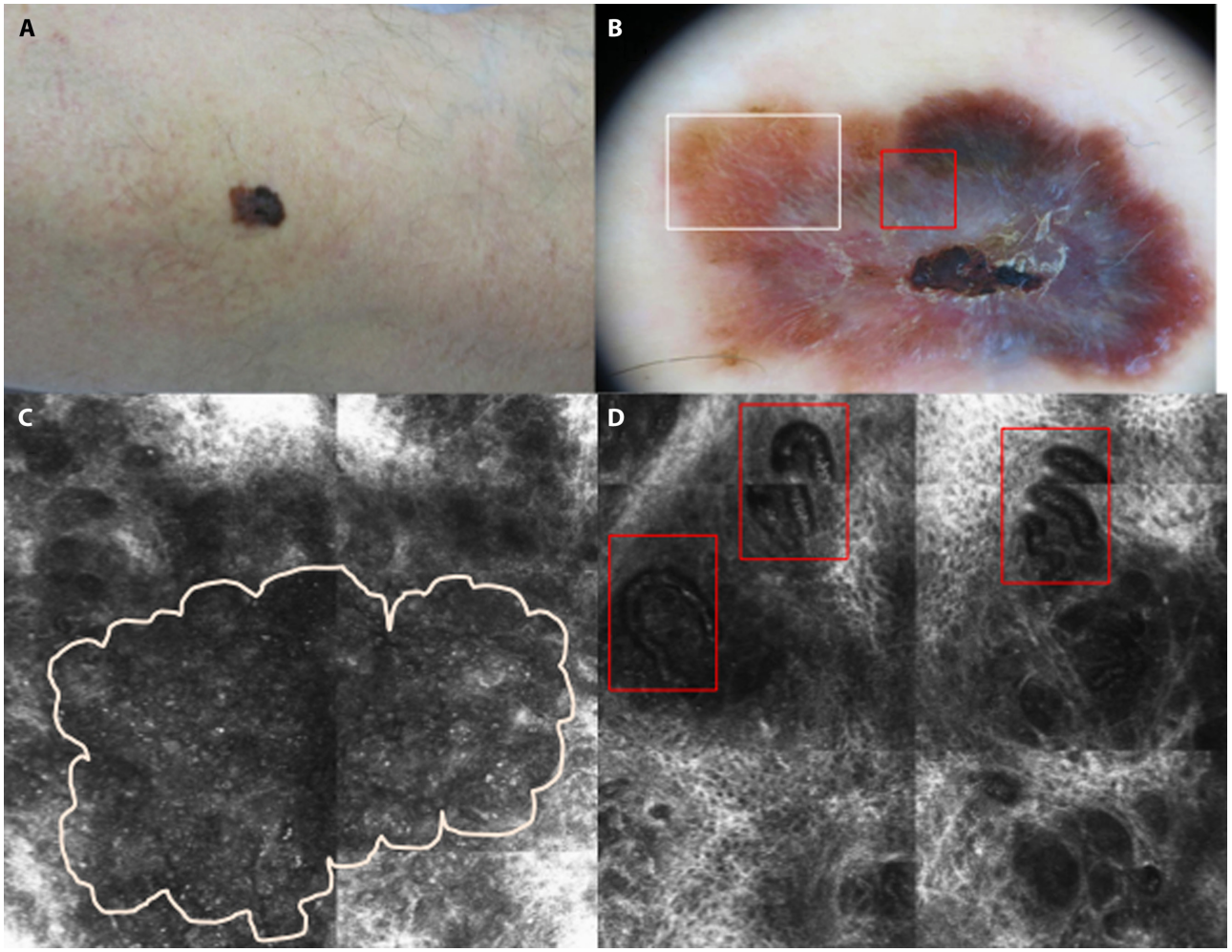

Figure 1. Dermoscopic and Confocal findings in primary melanoma. (A) Clinical appearance of pigmented asymmetric lesion on the leg. (B)Dermoscopic presence of asymmetric polychromic lesion, showing negative network (white square), bluewhite veil, atypical vessels (red square), ulceration and structureless pigmented areas. (C) Reflectance Confocal Microscopy (RCM) imaging (800micronsx800micros) at dermoepidermal junction (DEJ) and superficial dermis showing cerebriform nests with amorphous pleomorphic cells. Non edged papillae and atypical cells were visible at DEJ. (D) RCM imaging (1mmx1mm) at superficial dermis demonstrated multiple atypical high caliber vessels (red squares).

Atypical honeycomb was more frequently found in superficial spreading melanomas (SSMM) - seen in 65.2% while only in 37.5% of non-SSMM cases (P = 0.03). The presence of large atypical pagetoid cells and the dendritic shape of pagetoid cells were both more frequent in SSMM (60.7%, P = 0.01 and 59%, P = 0.05; respectively).

Importantly, confocal observation of dermal nests showed a mean increase of 1.6 mitosis over the mean mitotic rate

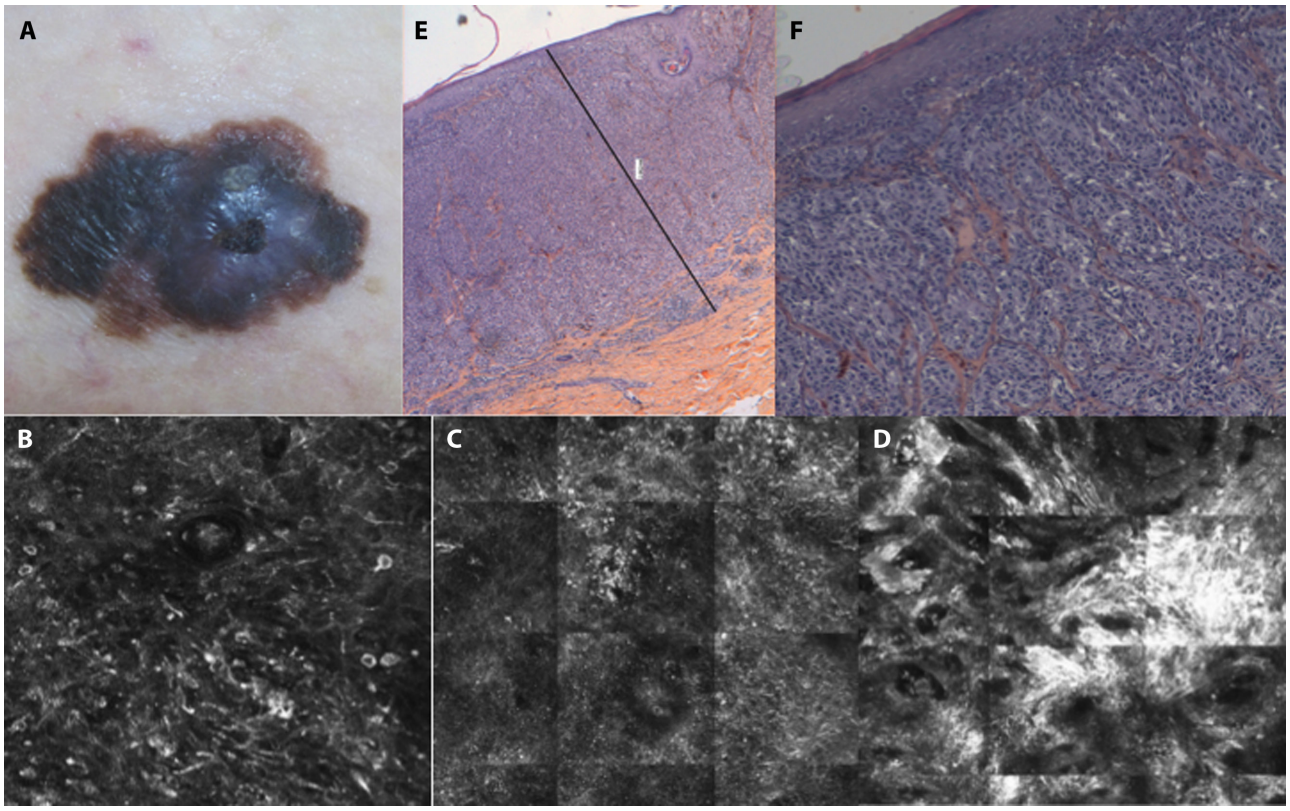

Figure 2. Confocal and histopathological correlation of primary melanoma. (A) Clinical image of superficial spreading ulcerated melanoma on the thigh of a lady in her 60s. (B) Reflectance confocal microscopy (RCM) single image (0.5 mm x 0.5 mm) at superficial epidermis layer, multiple dendritic and roundish pagetoid cells (combined type melanoma). (C) RCM mosaic imaging (1.5 mm x 1.5 mm) at suprabasal epidermal layer; multiple dendritic and roundish atypical cells. (D) RCM mosaic imaging (1.5mm x 1.5 mm) at DEJ and papillary dermis; atypical dendritic and pleomorphic cells at DEJ isolated and forming junctural nests and junctural thickenings, with non-edged papillae. (E) Histopathological (H&E) (x40) demonstrated ulcerated superficial spreading melanoma with vertical growth, Breslow 2mm. (F) Histopathological (H&E) (x100) correlated with nesting tendency and mitotic index of 10/mm^2.

(P = 0.006) and the presence of bright particles and plump cells (that is, diffuse inflammatory infiltrate in the superficial dermis) was associated with a mean increase of 0.6 mm in the Breslow index (P = 0.04) and a higher mitotic rate (P = 0.04). The presence of atypical nucleated cells within dermal papillae was strongly associated with a higher mitotic rate (P = 0.003). On the other hand, the presence of typical basal cells was related to a lower mitotic rate (P = 0.002).

As regards the confocal patterns, a dendritic confocal pattern was significantly associated with brisk tumour infiltrating lymphocytes (TILs) (29.4%, P = 0.021). The dendritic subtype tends to appear in tumors showing more histological regression, but the association was not significant, (P = 0.38). No other significant associations were found regarding the 4 melanoma confocal subtypes. Histological ulceration was not associated with any specific confocal pattern: it was observed in 2.4% dendritic confocal patterns (P = 0.16) and in 11% combined confocal patterns (P = 0.47).

Conclusions

The present study on 92 primary melanomas allowed the identification of in vivo features related to the main histological prognostic factors in localized melanoma. The in vivo predictors of high mitotic rate and tumor thickness are 1) dermoscopically: absence of pigment network and the presence of structureless pigmentation and vessels, and 2) confocal dermal presence of: diffuse inflammatory infiltrate, atypical nucleated cells and nests (related only to a higher mitotic rate).

Dermoscopy has previously been demonstrated to predict histologic features such as regression phenomena or melanoma depth [18-23]. Further, the presence of a brown atypical pigmented network has been associated with melanomas showing no mitoses [4]. In accordance with the literature, the present study validates the observation of a dermoscopic pigmented network as related to early melanomas (that is, with both lower Breslow index and mitotic rate). Additionally, we found that visualization of atypical vessels, blotches or structureless pigmentation are related to higher thickness.

In contrast to what we expected, the presence of dermoscopic ulceration and blue-white veil were not significantly associated with higher invasiveness or mitotic rate as reported by Deinlein et al [5]. Interestingly, in our series, there was a notable discrepancy between dermoscopic ulceration (present

in almost 20% of cases) and histopathologic ulceration (less than 7% were ulcerated in the definite report).

Interestingly, dermoscopy of primary melanoma has also been demonstrated as useful in predicting sentinel lymph node biopsy status [6]. There is a dermoscopic algorithm, which can achieve a sensitivity of 96.3% and a specificity of 52.1% ($P < 0.001$) for positive sentinel lymph node prediction.

On the other hand, molecular studies support the view that melanoma comprises distinct types of tumors and suggest that specific morphological features may help predict its clinical behavior. In line with this, and given some dermoscopic attributes of melanoma, such as features of regression and vascularity, were strongly linked to the overall amount of genomic damage [24].

In the present study, with the addition of confocal pre-surgical characterization, we demonstrated that a higher mitotic index is expected when dermal nests and atypical nucleated cells are found. In addition, a higher mitotic index and Breslow depth can be predicted when diffuse inflammatory infiltrate is observed in the superficial dermis.

As also described by Grazziontin et al in the setting of multiple primary and familial melanomas in our center, we could validate the confocal classification of sporadic melanomas into the 4 confocal types [19]. In our series the predominant confocal pattern was the dendritic cells subtype (44.6%) in contrast to the Pellacani et al cohort, which found a similar value for the combined type (47%). In our series the combined type was seen only in 9.8% of the 92 cases. However, this is in line with Grazziontin et al who found only 2 combined cases (4%) in a multiple and familial melanoma series of 57 cases from our center. This difference could be explained in part due to 1) the location of the present cohort of tumors (only trunk and limbs were included) and 2) in an attempt to avoid a large number of non-classifiable melanomas, we tried to classify all melanomas according to the predominant cell type present in more than 50% of the lesion, as done by Grazziontin et al [25]. On the other hand, Pellacani et al hypothesized that combined type could be a more advanced stage of other types, although in our series this could not be demonstrated, despite the fact that our mean Breslow (1.43 mm, SD 1.6) was higher than in their series (mean 0.79 mm, SD 0.99). Interestingly, melanoma RCM subtypes have been correlated with different profile expression markers, that could be suggestive of progression and an increase in aggressiveness, depending on RCM morphologies [26].

The main limitations of the present study include that it is a retrospective single-centre study, with a limited sample where only melanomas located on trunk and limbs were included. Further studies which combine different imaging techniques will allow better in vivo characterization of skin tumors, such as the complementation of RCM with optical coherence tomography, which has a deeper reach and a dynamic mode of vascular examination [29,30].

The relevance of this study lies in the identification of in vivo predictors of melanoma thickness and high mitotic rate by dermoscopically identifying the absence of pigment network and the presence of structureless pigmentation and vessels, and on RCM, the presence of nests and diffuse inflammatory infiltrate in the dermis. A more accurate prediction of prognosis prior to excision of a primary tumor would optimize the first approach to melanoma patients in terms of accurate indications for radiologic staging tests and for surgery. This preliminary study lays out a new approach to further characterization of early junctional phenomena, crucial for melanoma progression such as the study of TILs and environmental interaction between melanoma cells and host stroma.

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