SPITZ NAEVI AND MELANOMAS WITH SIMILAR DERMOSCOPIC PATTERN: CAN CONFOCAL MICROSCOPY DIFFERENTIATE?

Running head: SNs and MMs with similar dermoscopic pattern: the role of RCM

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Abstract

Background Differentiating Spitz naevi ~~(SNs)~~ and melanomas ~~(MMs)~~ can be difficult both clinically and dermoscopically. ~~In fact, it is common, in clinical practice, to find SNs simulating MMs, but also MMs simulating SNs.~~ Previous studies reported the potential role of *in vivo* reflectance confocal microscopy (RCM) in increasing diagnostic accuracy.

Objectives To define RCM criteria that can differentiate "false twins", namely SNs and MMs sharing similar dermoscopic appearance.

Methods Lesions histopathologically-diagnosed as ~~SNs~~Spitz naevi or ~~MMs~~melanomas were retrospectively retrieved and they been selected to cover all dermoscopy types and put in «couple». Lesions were classified in three main dermoscopic categories: globular and starburst pattern; spitzoid with dotted vessels; multicomponent or aspecific pattern.

Results RCM findings revealed that ~~marked~~ striking cell pleomorphism within epidermis, widespread atypical cells at the dermo-epidermal junction and marked pleomorphism within nests were significantly associated with the diagnosis of MM while spindled cell and peripheral clefting were exclusively found and pathognomonic of SNs. Furthermore, the analysis of dermoscopic subgroup highlight the importance of striking pleomorphism and spindled cells as clues to differentiate "false twins" with globular or starbust pattern.

Conclusions The current study highlights the role of RCM in discriminating "false twins" of ~~SNs~~Spitz naevi and ~~MMs~~melanomas ~~in adults, in particular~~

for lesions showing starburst and globular patterns upon dermoscopy whereas RCM is not useful in the other dermoscopic subgroups.

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Bulleted statements

what's already known about this topic?

The differential diagnosis between ~~SNs~~Spitz naevi and ~~MMs~~melanomas can be challenging. RCM represents an adds-on tool that improves diagnostic accuracy for skin cancer.

what does this study add?

RCM is useful to discriminate ~~SNs~~Spitz naevi and ~~MMs~~melanomas sharing the same dermoscopic pattern, namely globular or starburst pattern while it’s not helpful in other dermoscopic subgroups.

INTRODUCTION

In 1948 Sophie Spitz described 13 cases of what she called “juvenile melanoma,” underlining its presumably good prognosis because only one case of her series had proven fatal.[1] During the following forty years, however, the entity described by Sophie Spitz was thought to be completely benign, with metastasizing cases being intuitively considered as cases of melanomas simulating Spitz naevi (Spitzoid melanoma). [2,3]

Several attempts have been made to establish objective criteria that can reliably differentiate Spitz Naevi ~~(SNs)~~ and melanomas ~~(MMs)~~ on clinical, histopathological and molecular ground. [2-7]

Dermoscopy significantly improved the recognition of ~~SNs~~Spitz naevi[3] that can be roughly grouped in 4 main patterns, namely ~~starburst, globular, homogeneous, reticular and inverse network~~.[3] starburst or globular; negative network or superficial black network; reticular, homogeneous, complex, or multicomponent pattern and nonspecific pattern.[17] [8] As a general rule, a “typical” ~~SN~~Spitz naevus reveals a symmetric arrangement of colours and structures. Conversely, asymmetrically distribution of dermoscopic structures such as peripheral streaks, pseudopods, or globules are thought to be melanoma criteria.

However, ~~SNs~~Spitz naevi and ~~MMs~~melanomas may show overlapping dermoscopic pattern rendering them as “false twins”. Reflectance confocal microscopy (RCM) is a pivotal imaging tool that is used as an adjunct to dermoscopy for the diagnosis of melanocytic tumors.[8-14] [9-15]A correlation between dermoscopic findings, histopathologic

aspects and *in vivo* RCM features of ~~SNs~~Spitz naevi has been previously identified.[~~8,15-17~~ 8-9, 16-17]

In our study, we sought to identify distinctive RCM features that can potentially differentiate between “false twins”, namely, SNs and MMs typified by a similar dermoscopic pattern.

MATERIALS AND METHODS

Study population

Histopathologically-verified ~~SNs~~Spitz naevi and ~~MMs~~melanomas excised between 2010 and 2014 were retrieved from databases of 2 tertiary skin cancer centers (Modena and Reggio Emilia, Italy). Exclusion criteria were lesions located on special body sites such as acral and mucosal areas.

Instruments and criteria

Dermoscopy

Dermoscopic pictures were collected with DermLite Photo 3Gen (San Juan Capistrano, CA, US). Lesions were selected in order to put them in “couple” that shares similar dermoscopic aspect. Then they were classified, according to dermoscopy, in three main categories: 1. Globular and starburst pattern; 2. spitzoid with dotted vessels; 3. multicomponent or non-specific pattern. These two latter entities were grouped into the third category in order to obtain a larger group.

RCM evaluation

After the selection of couples of ~~SN~~Spitz naevus and ~~MMs~~melanoma with similar dermoscopic appearance, an accurate analysis of RCM was performed on the entire lesion area. RCM evaluation was carried out by means of a wide-probe confocal microscope (Vivascope 1500®, MAVIG GmbH, Munich, Germany), obtaining mosaics (Vivablock®) of high-resolution within the epidermis, at the dermal–epidermal junction (DEJ) and within the upper dermis; Vivastack® were also performed, following previously described procedures.~~[8,17]~~ [8-9]

One expert Investigator, different from the one who performed the image acquisition, analyzed all cases according to a list of RCM features[~~8-14,17-24~~8-15,18-24] (**Table 1 Supplementary**) blinded from histopathologic diagnosis or other information with the exception of body site. A second investigator was involved in case of uncertainty.

~~Histopathologic evaluation~~

~~Histopathologic evaluation was focused on the following criteria: epithelioid and/or spindled cell morphology, cell pleomorphism, pagetoid infiltration, epidermal hyperplasia, epidermal flattening and sharp borders.~~ Then, a side by side comparison between RCM and hematoxylin and eosin slides has been done according to our workup.[25]

Statistical analysis

Statistical evaluation was carried out with the SPSS® statistical package (SPSS Inc., Chicago, IL, USA).

Outcome dichotomous variable was set to definite RCM and histopathologic clues of ~~MM~~melanoma or ~~SN~~Spitz nevus. Demographic, clinical, and dermoscopic variables were included in the analysis. Absolute and relative frequencies of observations in benign and malignant lesions were described. Fisher exact test χ^2 was used to compare RCM ~~and histopathology~~ criteria among different type of lesions. A stratified analysis within each dermoscopic group was also carried out. Cohen's kappa coefficient was calculated in order to find association between confocal features and histology findings.

A *p*-value <0.05 was considered significant.

RESULTS

A total of 34 cases were included (10 were males and 24 females). Mean age was 49,59±16,42 for melanoma patients and 31±10,72 for subjects with Spitz naevi, ranging from 13 to 81 years.

Of 17 ~~MMs~~melanomas, 8 (47%) were *in situ* while 9 were invasive with a mean Breslow's thickness of 0.37 mm. None of the melanoma included was a spitzoid melanoma histologically. Lower limbs were the most frequent body location (18/34 lesions, 52,9%), followed by upper limbs (8/34, 23,5%), trunk (7/34, 20,6%) and only one case located on the head. ~~Analytic results of demographic information and~~

~~anatomic site of the tumor are reported in **Table 2**.~~ The dermoscopic appearance of the lesion of the head justified the enrollment in this study. No significant difference was found between ~~MMs~~melanomas and ~~SNs~~Spitz naevi for sex, age and body location.

Based on the dermoscopic analysis, 8 couples of ~~SNs/MMs~~Spitz naevi/melanomas (16/34 lesions, 47%) were identified within group 1 (**Figs 1-2**), 2 couples (4/34 lesions, 11,8%) within the second group (**Figs 3-4**) and 7 couples (14/34 lesions 41,2%) belonging to group 3 (**Figs 5-6**).

RCM evaluation

Several features were evaluated according to different skin depth (**Table ~~3~~2**). No significant differences were found for epidermal patterns between ~~SNs~~Spitz naevi and ~~MMs~~melanomas. Pagetoid cells were a common finding in both lesion type and were represented by dendritic/spindled cells more frequently than roundish in ~~SNs~~Spitz naevi (76,5% *versus* 23,5%, respectively). Hyporeflective pagetoid cells were observed in amelanotic tumors. ~~An higher quantity of pagetoid cells was found in 9 MMs compared to 5 SNs. Similarly, a~~ A widespread pagetoid infiltration occurred in 12 lesions, 9 malignant and 3 benign. A correlation between striking cell pleomorphism and ~~MM~~melanoma was established (p=0,010).

Evaluating the DEJ level, no significant association was observed between DEJ architecture and specific lesions group. Atypical cells were present in 14 ~~MMs~~melanomas *versus* 9 ~~SNs~~Spitz naevi. ~~SNs~~Spitz naevi

were often characterized by the presence of few atypical cells, predominantly spindled (p=0,003), showing numerous markedly atypical cells throughout the DEJ level in only 5 cases. Conversely, ~~MMs~~melanomas predominantly showed numerous (58,8%) atypical and pleomorphic cells, distributed throughout the entire lesion (11/17 p=0,037). Sharp borders were related with the diagnosis of benign lesions (p=0,044).

At the level of upper dermis, inflammation and dermal nests (dense, dense non-homogeneous) presented a similar distribution among ~~MMs~~melanomas and ~~SNs~~Spitz naevi while dense and sparse nests were exclusively described in ~~MMs~~melanomas (3/17). A marked cell pleomorphism within nests was significantly associated with ~~MMs~~ melanoma (p=0,003).

~~Histopathology evaluation~~

~~Histopathologic findings were are reported in **Table 4**. A statistically significant association between cell pleomorphism (p=0,007), pagetoid infiltration (p=0,026) and epidermal flattening (p=0,044) and MMsmelanoma was found while epidermal hyperplasia (p=0,044) and sharp borders (p=0,018) were significantly associated with SNsSpitz naevus.~~

~~Other interesting findings were the presence of spindled melanocyte in 47,1% of SNsSpitz naevi *vs* 11,8% of MMsmelanomas while epithelioid cells characterized 70,6% of SNsSpitz naevi *vs* 52,9% of MMsmelanomas.~~

~~***Dermoscopic group analysis***~~ ***RCM analysis within dermoscopic groups***

Group 1 revealed the presence of helpful RCM clues to differentiate ~~MMs~~melanomas and ~~SNs~~Spitz naevi. In details, a ~~marked~~ striking cell pleomorphism within epidermis was significantly associated with the diagnosis of ~~MM~~melanoma (p=0,041) while a few dendritic cells, with a prevalence of spindled cells, not involving the entire lesion area, were typically found in ~~SNs~~Spitz naevi (p=0,026). Furthermore, malignant lesions showed epidermal disruption, roundish pagetoid cells, many dendritic and atypical cells distributing throughout the entire lesion, junctional nests. A ringed pattern at the DEJ and peripheral clefting (being this latter exclusively found in ~~SNs~~Spitz naevi) were represented in one half of ~~SNs~~Spitz naevi.

RCM results regarding groups 2 and 3 were not statistically significant.

~~Within group 2 and 3, epidermal disruption, junctional nests, many dendritic and atypical cells distributed throughout the entire lesion were suggestive for a diagnosis of MMmelanoma while a few dendritic cells not involving the whole lesion represented the expression of a SNSpitz naevus.~~

~~Additionally group 3 was typified by the presence of roundish pagetoid cells and marked pleomorphism within nests in MMsmelanomas and ringed pattern and peripheral clefting in SNsSpitz naevi.~~

~~Acanthosis, dendritic cells and inflammation were equally represented in both categories.~~

~~Histopathology analysis within different dermoscopic groups revealed the presence of cell pleomorphism, significantly associated with MMmelanoma diagnosis for group 1 (p=0,007).~~ ~~Of note, analyzing lesions of the same group, pagetoid infiltration characterized MMsmelanomas whereas spindled cells and sharp boundary were commonly observed in SNsSpitz naevi.~~

~~In group 2, pagetoid infiltration and epidermis flattening were suggestive for the diagnosis of a malignant lesions while spindled cells, epidermal hyperplasia and sharp boundary (being these two latter observed exclusively in SNsSpitz naevi) characterized SNsSpitz naevi.~~

~~Similarly to previous findings, in MMsmelanomas belonging to group 3, pagetoid infiltration and epidermis flattening could be found and spindled cells in SNsSpitz naevi.~~

Specific RCM criteria were significantly correlated with histopathologic criteria, namely the presence of sharp borders cut-off (5 and 6 Spitz naevi in RCM and histopathology, respectively) (ƙ=0,675; p<0,05), spindled cells (8 Spitz naevi in RCM and 2 melanomas and 8 Spitz naevi in histology) (ƙ=0,398; p<0,05) and striking pleomorphism (9 melanomas and 2 Spitz naevi in RCM and 7 melanomas in histology) (ƙ=0,502; p<0,05) ~~(**Table 5**).~~

DISCUSSION

The differential diagnosis between ~~SNs~~Spitz naevi and ~~MMs~~melanomas may represent a diagnostic pitfall both for Dermatologists and Pathologists.

In vivo reflectance confocal microscopy (RCM) is a novel imaging technique enabling the visualization of epidermis, DEJ and papillary dermal structures at a *quasi*-histologic resolution, improving diagnostic accuracy for different skin lesions.[~~8-14,17-24~~8-15,18-24] Previous studies showed that the presence of sharp border cut-off, junctional nests and melanophages were the most relevant features for differentiating ~~SNs~~Spitz naevi from ~~MMs~~melanomas.[~~17~~8] However, a low specificity in differentiating ~~MMs~~melanomas and ~~SNs~~Spitz naevi was identified, due to the impossibility to explore the skin in depth, thus hampering the evaluation of mitoses and maturation with depth.[~~8,15-17~~ 8-9,16-17]

In the present study, specific RCM features statistically differed between ~~MMs~~melanomas and ~~SNs~~Spitz naevi, highlighting some differences within each dermoscopic group. The presence of a striking pleomorphism within epidermis and nests and atypical cells scattered throughout the whole lesion area were statistically significant and independently correlated with malignancy. In particular, cell pleomorphism within epidermis was observed within ~~MMs~~melanomas belonging to group 1, including starburst and globular lesions. On the other hand, ~~SNs~~Spitz naevi were characterized by the presence of sharp borders cut-off and spindled cells, these latter being prevalent in group 1. A previous study[~~17~~

[8]showed a similar rate of pleomorphism between ~~SNs~~Spitz naevi and ~~MMs~~melanomas while and the presence of atypical spindled cells in 30% of ~~MMs~~melanomas examined. These differences can be justified by lesions included and interobserver variation.

Ulceration and cerebriform clusters were not found because of the recruitment method of ~~MMs~~melanomas. In fact, 47% of ~~MMs~~melanomas included in this study were *in situ* and the mean Breslow's thickness was less than 1 mm (0.37 mm). Specifically, early spitzoid-looking ~~MMs~~melanomas are more likely to show dermoscopic characteristics enabling a dermoscopic match with ~~SNs~~Spitz naevi.

No RCM criteria were identified for group 2 and 3, including Spitzoid-dotted lesions and lesions with multicomponent or non-specific patterns, respectively. This can be related to the thickness of these lesions, impairing the evaluation of the deeper part of the tumor. Translated into clinical practice, it means that lesions displaying dotted vessels or non-specific pattern do not benefit of RCM imaging and thus, other factors, such as clinical context and dermoscopy, should be considered for their proper management.~~16~~17

Results of histopathologic revision supported in part RCM findings. In fact, within the first group cell pleomorphism characterized ~~MMs~~melanomas while sharp borders were associated with ~~SNs~~Spitz naevi. ~~Furthermore, clues that cannot be easily studied with RCM were found. In particular, epidermal hyperplasia in SNsSpitz naevi and pagetoid infiltration, epidermal flattening in MMsmelanomas.~~ Some ~~of these~~

histology criteria showed an overlap with RCM findings: sharp borders, cell pleomorphism and spindled cells.

Demographic data revealed that all the lesions included in the study were found in patients older than 12 years of age. Intuitively, studying lesions analyzed by pathologists means including doubtful spitzoid lesions that are most common in patients older than 12 year-old. [17]

The main limitation of this study was the low number of cases selected that is related to the careful and detailed matching of difficult-to-diagnose tumors revealing spitzoid-looking pattern.

Clinical practice highlights the high variability of spitzoid lesions. Our study underlines the need of a careful examination of all spitzoid-looking lesions by combining dermoscopy with RCM to establish an accurate diagnosis for “false twins” and manages them accordingly. It supports the role of RCM in discriminating spitzoid lesions revealing a globular and starburst pattern ~~on dermoscopy~~, representing the most common dermoscopic patterns of SNs.[5] Further studies with a larger number of lesions are warranted in order to confirm our preliminary results.

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Figure legends

Figure 1. (**a**) Dermoscopy of a starburst melanoma, 0.63 mm Breslow thickness. (**b**) High magnification confocal microscopy showing several dendritic and roundish pagetoid cells at epidermal level (~~arrows~~ circle). (**c**) Corresponding histology presenting pagetoid infiltration and pleomorphic cells (~~arrow~~ circle) (hematoxylin-eosin stain; original magnification: 200X).

Figure 2. (**a**) Dermoscopy of a starburst Spitz nevus. (**b**) ~~Confocal mosaic~~ High magnification confocal microscopy at dermoepidermal junction showing sharp border cut-off (green line), a peripheral nest (asterisk) atypical cells with focal distribution in an acanthotic lesion. (**c**) Corresponding histology presenting a peripheral nest (asterisk) and sharply demarcated border (arrow) (hematoxylin-eosin stain; original magnification: 200X).

Figure 3. (**a**) Dermoscopy of a spitzoid-dotted melanoma, 0.5 mm Breslow thickness. (**b**) High magnification confocal microscopy showing hyporeflective, large and well-demarcated structures (arrows) within ~~the honeycombed pattern~~ epidermis. (**c**) Corresponding histology presenting non-pigmented pagetoid cells scattered throughout the epidermis (hematoxylin-eosin stain; original magnification: 200X).

Figure 4. (**a**) Dermoscopy of a spitzoid-dotted Spitz nevus. (**b**) High magnification confocal microscopy showing concentric honeycombed structures centered by thin dermal papillae (asterisks). (**c**) Corresponding

histology presenting an acanthotic epidermis with elongated cristae separated by thin papillae and hypopigmented melanocytes predominantly clustered in small nests at tip of epidermal cristae (hematoxylin-eosin stain; original magnification: 200X).

Figure 5. (**a**) Dermoscopy of a multicomponent melanoma, 1.06 Breslow thickness. (**b**) High magnification confocal microscopy showing nonhomogeneous nests with ill defined contours (asterisks). (**c**) Corresponding histology presenting pleomorphic melanocytes predominantly clustered in small nests (asterisks) (hematoxylin-eosin stain; original magnification: 200X).

Figure 6. (**a**) Dermoscopy of a multicomponent Spitz nevus. (**b**) High magnification confocal microscopy showing fusiform cells with bipolar elongations or dendrites (arrows). (**c**) Corresponding histology showing spindled melanocytes (arrows) (hematoxylin-eosin stain; original magnification: 200X).

TABLES

Table 1 Supplementary. List and definition of the RCM features evaluated for MMs and SNs.

RCM criteria	Definition
Epidermis	
Regular honeycombed pattern	large polygonal cells with dark nuclei and bright cytoplasm and cell borders
Regular cobblestone pattern	small polygonal cells with refractive cytoplasm separated by a less refractive border
Atypical honeycombed pattern	irregularity in size of the cells and thickness of the contour within a honeycombed structure
Atypical cobblestone pattern	irregularity in size and/or refractivity of the cells within a cobblestone structure
Epidermal disruption	disarray of the normal architecture of superficial layers with unevenly distributed bright granular particles and cells, in absense of honeycombed or cobblestone pattern
Acanthosis	prominent bright epidermis intermingled with papillae
Ulceration	dark areas, usually with sharp borders and irregular contours, filled with amorphous material and/or clotted bright small particles
Pagetoid cells	large roundish nucleated cells, twice the size of basal keratinocytes, with a dark nucleus and bright cytoplasm
Dendritic pagetoid cells	large cells with bright cytoplasm and dark nucleus with clearly visible dendrites connected to the cell
Roundish pagetoid cells	large bright cells with well outlined border and dark nucleus within the epidermis, represent the most common finding for melanoma diagnosis
Number of pagetoid cells	more than 5 clearly visible pagetoid cells evaluated on five samples of 0.5x0.5mm image
Widespread pagetoid cells	pagetoid cells scattered throughout the whole lesion area
Striking pleomorphism	variability of the aspect of pagetoid cell and/or the presence of cells with bizarre shapes
DEJ	
Ringed pattern	densely packed bright rings corresponding to papillae surrounded by a rim of small bright cells sharply contrasting with the dark background
Junctional nests	compact, round to oval bright cell aggregates, connected with the basal layer of the epidermis

Sheet-like strucure	cells distributed at the transition of the DEJ showing loss of dermal papillae not aggregated in clusters but closely distributed in the same plane with the loss of dermal papillae
Edged papillae	dermal papillae demarcated by a rim of bright cells, appearing as bright rings sharply contrasting with the dark background
Non-edged papillae	dermal papillae without a demarcating bright rim at the DEJ
Atypical cells	large cells showing a bright cytoplasm with clearly outlined borders and sharply contrasted dark nucleus inside, roundish to oval in shapes, sometimes presenting dendritic-like structures
Spindled cells	elongated cells oriented toward the same direction
Number of atypical cells	more than 5 clearly visible atypical cells evaluated on five samples of 0.5x0.5mm image
Widespread atypical cells	atypical cells scattered throughout the whole lesion area
Peripheral Clefting	dark slit-like space observed between tumor and surrounding skin
Upper dermis	
Dermal dense nests	compact aggregates with sharp margin in which outline of individual cells is indiscernible or similar in shape, size and refractivity
Dermal dense nonhomogenous nests	compact cell aggregates showing non homogeneity in cell morphology and reflectivity
Dense and sparse dermal nests	cell aggregates with irregular, discohesive margins showing isolated nucleated cells at the periphery
Cerebriform clusters	confluent amorphous brain-like aggregates of low reflecting cells exhibiting granular cytoplasm without evident nuclei and ill-defined borders, showing a fine hyporeflective “fissure” like appearance
Marked pleomorphism within nests	nonreflecting structures with a well-demarcated border, containing isolated round to oval cells with dark nucleus and reflecting cytoplasm, with bizarre shapes
Inflammation	large irregularly shaped bright cells with ill-defined borders and usually no visible nucleus which can be visible within dermal papillae

Table 2. Descriptive demographic and clinical characteristics of the study population.

-	**MM (17) N, (%)**	**SN (17) N, (%)**	***p*-value**
Age	-	-	-
Mean	49,5±16,4	31±10,7	-
-	-	-	-
Age group	-	-	-
<40	6 (35,3)	12 (70,6)	0,084
≥40	11 (64,7)	5 (29,4)	-
-	-	-	-
Sex	-	-	-
female	10 (58,8)	14 (82,4)	0,259
Male	7 (41,2)	3 (17,6)	-
-	-	-	-
Breslow	-	-	-
-	0,38±0,45	-	-
-	-	-	-
Location	-	-	-
head and neck	1 (5,9)	0	0,47
Trunk	4 (23,5)	3 (17,6)	-
upper limbs	5 (29,4)	3 (17,6)	-
lower limbs	7 (41,2)	11 (64,8)	-
-	-	-	-
Dermoscopic group	-	-	-
1	8 (47,1)	8 (47,1)	-
2	2 (11,8)	2 (11,8)	-
3	7 (41,1)	7 (41,1)	-

*p-value<0,05

Table ~~3~~ 2. List of RCM features discriminating MMs and SNs (p<0.05).

RCM criteria	MM (17) N, (%)	SN (17) N, (%)	*p*-value
Epidermis			
Regular honeycombed pattern	1 (5,9)	1 (5,9)	1
Regular cobblestone pattern	0	0	§
Atypical honeycombed	7 (41,2)	12 (70,6)	0,166
Atypical cobblestone	2 (11,8)	1 (5,9)	1,000
Epidemis disruption	7 (41,2)	3 (17,6)	0,259
Acanthosis	14 (82,4)	15 (88,2)	1,000
Ulceration	0	0	§
Dendritic pagetoid cells	14 (82,4)	13 (76,5)	1,000
Roundish pagetoid cells	10 (58,8)	4 (23,5)	0,080
Several pagetoid cells	9 (52,9)	5 (29,4)	0,296
Widespread pagetoid cells	9 (52,9)	3 (17,6)	0,071
Striking pleomorphism	9 (52,9)	2 (11,8)	0,010*
DEJ			
Ringed pattern	5 (29,4)	9 (52,9)	0,296
Junctional nests	13 (76,5)	9 (52,9)	0,282
Sheet-like structure	5 (29,4)	2 (11,8)	0,398
Edged papillae	11 (64,7)	9 (52,9)	0,728
Non-edged papillae	4 (23,5)	5 (29,4)	1,000
Atypical cells	14 (82,4)	9 (52,9)	0,141
Spindled cells	0	8 (47,1)	0,003*
Many atypical cells	10 (58,8)	7 (41,2)	0,494
Widespread atypical cells	11 (64,7)	5 (29,4)	0,037*
Peripheral clefting	0	5 (29,4)	0,044*
Upper dermis			
Regular dense dermal nests	6 (35,3)	6 (35,3)	0,601
Non-homogeneous dense nests	5 (29,4)	3 (17,6)	0,688
Dense and sparse dermal nests	3 (17,6)	0	0,227
Cerebriform clusters	0	0	§
~~Striking~~ Marked pleomorphism within nests	8 (47,1)	0	0,003*
Inflammation	16 (94,1)	14 (82,4)	0,601

*P-value <0,05
§no statistics was computed because the feature is a constant

Table 4. List of histopathologic criteria that discriminate MMs and SNs (p<0.05).

Histopathology criteria	***p*-value**
epithelioid cells	0,282
spindled cells	0,057
pleomorphism	0,007*
pagetoid spread	0,026*
epidermal hyperplasia	0,044*
epidermis flattening	0,044*
sharp boundary	0,018*

**p*-value <0,05

Table 5. RCM findings correlated with histologic features for MM and SN diagnosis ($p<0.05$).

Hystopathology	RCM	value	p
sharp boundary	peripheral clefting	0,675	<0.05
cell pleomorphism	striking cell pleomorphism	0,502	<0.05
spindled cells	spindled cells	0,398	<0.05