In Vivo Confocal Microscopic and Histopathologic Correlations of Dermoscopic Features in 202 Melanocytic Lesions

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Objectives: To identify in vivo microscopic substrates of the dermoscopic patterns of melanocytic lesions and to correlate them with histopathologic features.

Design: Before excision, lesion areas that showed characteristic dermoscopic patterns were imaged by dermoscopy and confocal microscopy and directly correlated with histopathologic features.

Setting: Departments of Dermatology of the University of Modena and Reggio Emilia and Hospital Clínic of Barcelona, between July 2006 and March 2007.

Patients: Patients with 202 melanocytic lesions, corresponding to 76 melanomas, 114 nevi, and 12 Spitz or Reed nevi.

Main Outcome Measures: Correlation of dermoscopic patterns in melanocytic lesions with confocal microscopic findings and conventional histopathologic findings.

Results: Characteristic architectural and cytologic substrates were identified in vivo with the use of confocal microscopy and correlated with histopathologic features. Pigment network atypia was evidenced through confocal microscopy as a disarrangement of dermoepidermal junction architecture and cellular atypia. Pigmented globules consisted of cell clusters, corresponding to melanocytic nests identified on histopathologic analysis. Black dots correlated with intraepidermal reflective spots or with large pagetoid cells in nevi and melanoma, respectively. Blue structures usually consisted of numerous pleomorphic cells, corresponding to malignant melanocytes and inflammatory cells in melanomas, whereas plump bright cells, corresponding to melanophages on histopathologic analysis, characterized benign lesions. Within regression, a retiform distribution of collagen fibers, which sometimes intermingled with melanophages and rarely with nucleated cells, was observable.

Conclusions: The knowledge of the cytologic and architectural aspects of the different dermoscopic patterns, as they appear by in vivo confocal microscopy, may guide the user to the identification of specific substrates in melanocytic lesions and consequently the interpretation of the dermoscopic features.

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DERMOSCOPY IS A WIDELY diffused technique based on the visualization and magnification of subsurface structures, which is useful for diagnostic purposes. The identification of specific structures leads the observer to the diagnostic definition of melanocytic and nonmelanocytic skin lesions.1-4 Although some dermoscopic features correspond to histologic findings, a direct and exact correlation is difficult to determine. A step-sectioning procedure5,6 and micropunch technique7 were used to correlate accurately dermoscopic features with their histopathologic counterparts. Moreover, horizontal histologic sections can be performed; however, this procedure is limited to selected features because of the risk of interference with the Breslow thickness evaluation.8

In vivo reflectance confocal microscopy (RCM) produces horizontal images of the skin at a cellular level resolution from the surface to the papillary dermis. It has been used for the study of normal skin and different diseases,9,10 resulting in the differential diagnosis of malignant melanoma (MM).11-13 Because the single image on the monitor during patient examination corresponds to a small area of approximately 0.5 mm per side at a precise depth, the interpretation of the confocal features depends strongly on the knowledge of the structure under examination. Correlations among some dermoscopic features, such as pigment network, globules, streaks, and blue structures, as determined by confocal microscopy and histologic analysis have recently been shown.14-20

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Confocal microscopy seems to be the natural link between dermoscopy and histopathologic analysis because of its high resolution, horizontal imaging, and non-invasiveness. Therefore, we aimed to identify RCM aspects that correspond to dermoscopic features of melanocytic lesions and to correlate them with histopathologic features, systematically exploring all the observable features of melanocytic lesions in a large series of cases.

The study has been approved by the institutional review boards of the University of Modena and Reggio Emilia and Hospital Clínico of Barcelona, and the Declaration of Helsinki protocols were followed. Participants gave their written informed consent.

METHODS

This study included a total of 202 melanocytic lesions from as many patients who were recruited at the Department of Dermatology of the University of Modena and Reggio Emilia and at the Melanoma Unit of the Hospital Clínico of Barcelona, between July 2006 and March 2007 following the same protocol. The lesions corresponded to 76 MMs (22 in situ, 33 thinner than 1 mm, 14 with a thickness between 1 and 2 mm, 7 with a thickness of >2 mm), 114 melanocytic nevi (33 junctional, 70 compound, 8 intradermal, and 3 blue nevi), and 12 Spitz or Reed nevi, consecutively recorded because each dermoscopic feature was observed in at least 15 cases. Lesions were from the lower limbs in 43 cases, upper limbs in 19, chest in 28, abdomen in 24, and back in 88. No lesion from the face, palms and soles, mucosal areas, and genital regions was considered for the peculiarity of dermoscopic patterns. When centering the RCM adapter ring onto the lesion area of interest, a direct correlation among dermoscopic, confocal microscopic, and histopathologic features was obtained in all cases. Digital dermoscopic and RCM images of the ring-delimited area were acquired, and a silk suture or an ink mark at 1 pole of the specimen was positioned in all cases to make its orientation easier. All lesions were then excised, and step sections were cut for diagnostic confirmation and pattern correlation. Common nevi included in this study, which had typical dermoscopic features, were excised only for cosmetic reasons and because of a patient request. Moreover, in cases in which the dermoscopic feature of interest was limited to a small area, a 2-mm punch incisional biopsy in that particular area of the lesion was also performed to obtain a perfect histopathologic correlation.

INSTRUMENTS

Digital dermoscopy imaging was performed by high-resolution digital dermoscopes (FotoFinder; TeachScreen Software GmbH, Bad Birnbach, Germany; and DermLite Foto; 3GEN LLC, Dana Point, CA) for all cases. Moreover, in some cases a low-resolution dermoscopic camera integrated into the Vivascope software was used to allow precise confocal navigation and to look at dermoscopic images (VivaCam; Lucid Inc, Henrietta, New York, NY).

The RCM images were acquired by means of near-infrared reflectance confocal laser scanning microscopes (Vivascopes 1000 and Vivascope 1500; Lucid). Instruments and acquisition procedures are described elsewhere.4,9 A minimum area of \(4 \times 4 \) mm was acquired (block image) to visualize the corresponding area by means of dermoscopy. Subsequently, series of high-resolution images (capture and stack images) were obtained at different levels from the surface down to the papillary dermis. Each image corresponds to a horizontal section at a selected depth with a \(475 \times 350\)-µm field of view for Vivascope 1000 and a \(500 \times 500\)-µm field of view for Vivascope 1500.

Histologic pictures were acquired by means of a light microscope (Axioskope 40; Zeiss, Göttingen, Germany) equipped with a digital camera (AxioCam MRc5; Zeiss) and dedicated software.

IMAGE DESCRIPTION

Dermoscopic image description of each pattern was performed according to the definition of the literature.1,2,4-6 The RCM images were described using the terms previously proposed and recently summarized in a consensus terminology glossary.11,13,21,22 Histopathologic description of both architectural patterns and cytologic features was performed on several hematoxylin-eosin–stained 4-µm sections derived from the block corresponding to the dermoscopic and confocal area of interest. Histologic correlations were noted and confirmed by studying several similar cases.

RESULTS

The most relevant confocal aspects and their histologic substrates for each dermoscopic feature are listed in the Table.

PIGMENT NETWORK

Fifty-two lesions presented with a typical pigment network, all corresponding to melanocytic nevi, whereas an atypical pigment network was observed in 28 MMs (8 in situ, 19 thinner than 1 mm, and 1 thicker) and 27 nevi. When using RCM, a typical network was characterized in all cases by rings of bright polygonal cells surrounding roundish to oval dark areas corresponding to dermal papillae, observable at the dermoepidermal junction between 60 and 100 µm below the surface. The size and shape of the dermal papillae exactly corresponded to the ones of the network holes, whereas the network lines were correlated with 2 paired portions of adjacent rings, resulting at high-magnification dermoscopy in the bilayer structure of the network grid.22 Routine histologic analysis revealed elongated rete ridges with an increased number of melanocytes in the basal layer and pigmented basal keratinocytes (Figure 1). A club-shaped elongated rete ridge with prominent basal layer pigmentation along with larger melanocytes was observed in 2 cases of junctional nevi that exhibited a lentiginous pattern, compatible with the larger size of the bright cells forming the rings on confocal microscopy. No pagetoid melanocytosis or single or clustered atypical cells were present on RCM and histologic analysis. In 8 cases, a few plump bright cells were visible within the dermal papilla, corresponding to small bluish structures within the holes of the network and melanoocytes on histologic analysis.

The atypical pigment network corresponded to irregular and dishomogeneous dermal papillae. These papillae did not have a demarcated rim of bright cells but were separated by loosely thick interpapillary spaces. These spaces consisted of large reflecting cells that coincided with the irregular network grid, previously defined as "nonedged papillae,"10 on RCM. Irregular enlargements of the intrapapillary spaces were described in 19 MMs and 19 nevi. Cells were usually larger than the typical keratinocytes, although atypical cells, with highly...
Histopathologic examination revealed a disarrangement observable in 27 of 28 MMs and 4 of 27 nevi on RCM. A presence of roundish pagetoid cells, large atypical cells at the dermoepidermal junction, nucleated cells infiltrating papillary dermis, and/or clusters of pleomorphic cells.

Table. Confocal Microscopic and Histopathologic Correlations of Dermoscopic Features in Melanocytic Lesions

<table>
<thead>
<tr>
<th>Dermoscopic Pattern</th>
<th>No. of Cases</th>
<th>RCM Features</th>
<th>Frequency of RCM Atypia, No. (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigment network</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typical</td>
<td>52</td>
<td>Edged papillae: rings of bright cells surrounding roundish to oval dark areas corresponding to dermal papillae at the dermoepidermal junction.</td>
<td>0 (0) Elongated regular rete ridges with an increased number of melanocytes in the basal layers and pigmented basal keratinocytes.</td>
</tr>
<tr>
<td>Atypical</td>
<td>27 27</td>
<td>Nonedged papillae: dark dermal papillae usually irregular in size and shape, without a demarcated rim of bright cells, but separated by more or less thick interpapillary spaces constituted by large reflecting cells coinciding with the irregular network grid.</td>
<td>27 (96) 4 (15) Disarrangement of the rete ridge in all cases. Increased number of atypical melanocytes predominantly in melanomas.</td>
</tr>
<tr>
<td>Pigment globules</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular</td>
<td>15</td>
<td>Dense melanocytic clusters: compact aggregates with sharp margins, constituted by large polygonal monomorphous cells.</td>
<td>0 (0) Typical and monomorphous nevocytic nests at the dermoepidermal junction and within the papillary dermis.</td>
</tr>
<tr>
<td>Irregular</td>
<td>27 20 3</td>
<td>Irregularly shaped clusters, usually showing regular cytologic features in benign lesions and atypical cells in melanomas.</td>
<td>22 (81) 4 (20) 2 (67) Typical nevocytic nests corresponded to homogeneous dense clusters, whereas nonhomogeneous clusters showed up as compact aggregates of pleomorphic melanocytes.</td>
</tr>
<tr>
<td>Pigment dots</td>
<td>61 71 8</td>
<td>Single large pagetoid cells, frequently aggregated in small clusters, were predominantly observable in MMs and some Spitz nevi, whereas bright structures within an even epidermis were typical of benign lesions. Blush dots corresponded to plump bright cells within papillary dermis.</td>
<td>52 (81) 9 (13) 5 (63) Cells in superficial layers corresponded to pagetoid infiltration, whereas bright structures showed up as free melanin clumps within the epidermis. Plump bright cells corresponded to melanophages.</td>
</tr>
<tr>
<td>Streaks</td>
<td>27 17 8</td>
<td>Radial streamings: parallel series of elongated lines of basal cells projected toward the periphery. Peripheral globules: dense clusters at the lesion periphery. Pseudopods: globular-like bulging structures, similar to dense nests bridged with the lesion core.</td>
<td>4 (15) 0 1 (13) Radial streamings: elongated and parallel oriented epidermal crista at the periphery of the lesion. Peripheral globules: typical nevocytic nests at the periphery. Pseudopods: well-defined nests, located at the tip of the enlarged and parallel oriented crista.</td>
</tr>
<tr>
<td>Diffuse pigmentation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light brown</td>
<td>36</td>
<td>Regular honeycombed pattern.</td>
<td>0 (0) Few pigmentation and regular epidermal architecture.</td>
</tr>
<tr>
<td>Dark diffuse</td>
<td>36 18 5</td>
<td>Bright cobblestone pattern, sometimes intermingled with cells spreading upward in melanomas and Spitz nevi.</td>
<td>31 (58) 2 (11) 3 (60) Pigmentation within keratinocytes and transdermal melanin loss together with pagetoid melanocytosis in correspondence with spreading upward cells.</td>
</tr>
<tr>
<td>Pigment and pigment blots</td>
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<td></td>
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<tr>
<td>Blue structures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue-whitish veil</td>
<td>20 1 1</td>
<td>Disarranged pattern and presence of roundish pagetoid infiltration in superficial layers, nonedged papillae and cytologic atypia in basal layer, dishomogeneous and/or cerebriform nests and nucleated and/or plump cells in dermal papillae.</td>
<td>10 (100) 0 1 (100) Orthokeratosis and parakeratosis associated with pagetoid melanocytosis. Disarray of the dermoepidermal contour, marked cell atypia, and malignant cells in nests, cords, or solitary infiltrating the dermis together with melanophages and inflammatory cells.</td>
</tr>
<tr>
<td>Blue areas and blue diffusion</td>
<td>3 10 2</td>
<td>Plump bright cells within dermal papillae. Blue nevi: no specific features.</td>
<td>3 (100) 0 2 (100) Melanophages and inflammatory infiltrate in the dermis. Blue nevi: ill-defined deep dermal proliferation of elongated and dendritic dermal melanocytes.</td>
</tr>
<tr>
<td>Regression</td>
<td>34 3</td>
<td>Thin epidermis and coarse network of ill-defined grany bundles or fibers in the dermis, sometimes intermingled with small bright reflecting spots and plump bright cells.</td>
<td>7 (21) 0 (0) Thin, devoid of melanin, epidermis, covering areas of fibroplasia with inflammatory infiltrate, constituted by leukocytes and few melanophages.</td>
</tr>
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</table>

Abbreviations: MM, malignant melanoma; RCM, reflectance confocal microscopy.

<sup>a</sup>Presence of roundish pagetoid cells, large atypical cells at the dermoepidermal junction, nucleated cells infiltrating papillary dermis, and/or clusters of pleomorphic cells.

Reflective cytoplasm and occasionally with branching dendritic-like structures, located at the dermoepidermal junction and/or spreading upward in a pagetoid fashion, were observable in 27 of 28 MMs and 4 of 27 nevi on RCM. Histopathologic examination revealed a disarrangement of the rete ridge in all cases, whereas in all MMs and only 2 nevi an increased number of atypical melanocytes, sometimes forming irregular and confluent nests at the dermoepidermal junction, were observable (Figure 2).
PIGMENT GLOBULES

Regular homogeneous globules were studied in 15 nevi, 5 of which corresponded to dermal nevi. An exact correspondence in shape was observed between the brown globules on dermoscopy and the dense melanocytic clusters on RCM, appearing as compact aggregates with a sharp margin of large polygonal cells similar in morphologic features and reflectivity. Histopathologic analysis revealed discrete melanocytic nests, composed of typical and monomorphous nevocytes, located at the dermoepidermal junction and within the papillary dermis (Figure 3 and Figure 4). The large polygonal structures forming the dermoscopic cobblestone pattern in the 5 dermal nevi appeared on RCM as large aggregates of clustered cells, with enlarging dermal papillae and without connection to the basal cell layer. Some large, roundish nucleated cells loosely aggregated were sometimes visible in the upper portion of the cluster. In depth, clusters assumed a more compact and homogeneous aspect with no evident cell contours. On histologic examination, nevus cells were disposed in an orderly manner as cords and nests of cells decreasing in size with depth and separated by thin fibers (Figure 5).

(A, B, C)
Globules, irregular in size, shape, pigmentation, and/or distribution, were studied in 27 MMs, 20 melanocytic nevi, and 3 Spitz or Reed nevi. The RCM revealed irregularly shaped clusters in all lesions, with a regular dense structure immediately below the dermoepidermal junction (white arrows) (B) (original magnification ×30), corresponding to melanocytic nests in the papillary dermis on histologic analysis (white arrowheads) (C) (hematoxylin-eosin, original magnification ×200).

Pigment dots were observed in 140 lesions, belonging to 61 MMs, 71 nevi, and 8 Spitz or Reed nevi. In the superficial layers of 52 of 61 MMs, single large cells, frequently aggregated in small clusters, with reflective cytoplasm and dark nucleus spreading upward in a pagetoid fashion, were clearly visible and highly contrasted in respect to the honeycombed or cobblestone background structure of the epidermis, showing up as small aggregates of pagetoid melanocytes on histologic analysis (Figure 6).

Figure 4. Histologic analysis revealed discrete melanocytic nests, composed of typical and monomorphous nevocytes, located at the dermoepidermal junction and within the papillary dermis. A, Dermoscopy of a compound nevus showing regular polygonal globules forming a cobblestone pattern (white dotted square corresponds to the reflectance confocal microscopy [RCM] image) (original magnification ×50). The RCM showed roundish to oval, large dense clusters immediately below the dermoepidermal junction (white arrows) (B) (original magnification ×30), corresponding to melanocytic nests in the papillary dermis on histologic analysis (white arrowheads) (C) (hematoxylin-eosin, original magnification ×200).

Figure 5. Histologic analysis revealed orderly nevus cells that were disposed as cords and nests of cells decreasing in size with depth and separated by thin fibers. A, Dermal nevus with a cobblestone pattern on dermoscopy (white dotted square corresponds to the reflectance confocal microscopy [RCM] image) (original magnification ×30). B, The RCM showed large, roundish, loosely aggregated cells in the upper portion of the cluster (white arrows) (original magnification ×30). C, On histologic analysis, nevus cells were disposed in an orderly manner as cords and nests of cells separated by thin fibers clearly visible in the superficial dermis, decreasing in size with depth (white dotted line corresponds to the level of the RCM image) (hematoxylin-eosin, original magnification ×100).

Figure 6. Histologic analysis revealed clusters of pleomorphic malignant melanocytes, predominantly distributed at the dermoepidermal junction and in the papillary dermis. A, Atypical pigment globules in a melanoma on dermoscopy (white dotted square corresponds to the reflectance confocal microscopy [RCM] image) (original magnification ×20). Irregularly shaped clusters, composed of nonhomogeneous morphologic features and reactivity cells, were visible using RCM (white arrows) (B) (original magnification ×30) and corresponded to atypical clusters of pleomorphic melanocytes at histologic analysis (white arrowhead) (white dotted line corresponds to the level of the RCM image) (C) (hematoxylin-eosin, original magnification ×100).
sis (Figure 7). On the other hand, 5 of 8 Spitz nevi showed few pagetoid cells and some ovoidal, homogeneously bright structures, corresponding to free melanin clumps within the stratum corneum. Pagetoid cells were also visible in 9 nevi, whereas the remaining cases showed a honeycombed and/or cobblestone pattern with the focal presence of reflecting spots on RCM, corresponding to melanin clumps within the epidermis on histologic analysis (Figure 8). On the other hand, bluish dots observed in 31 nevi were composed of small aggregates of plump bright cells within dermal papillae visible by RCM (Figure 9).

Streaks (peripheral structures) were present in 52 cases, 15 of which (8 MMs, 4 nevi, and 3 Spitz or Reed nevi) showed radial streamings, 19 (8 MMs, 8 nevi, and 3 Spitz or Reed nevi) peripheral globules, and 18 (11 MMs, 5 nevi, and 2 Spitz or Reed nevi) pseudopods. On RCM, radial streamings consisted of parallel series of elongated junctional thickening–like structures or of lines of interpapillary basal cells projected toward the periphery, separated by narrow elongated darker areas corresponding to dermal papillae. On histologic analysis, elongated and parallel oriented epidermal cristae were observable at the periphery of the lesion (Figure 10).
On the other hand, peripheral globules did not differ from brown globules, corresponding to dense or sparse cell clusters on RCM, exactly fitting in shape with dermoscopic findings. Melanocytic nests at the dermoepidermal junction, sometimes pushing up the epidermis, were observable on histologic analysis (Figure 11). In 4 of 8 MMs and in 1 of 3 Spitz or Reed nevi, irregular peripheral globules corresponded to aggregates of pleomorphic malignant melanocytes on histologic analysis (Figure 12).

With the use of RCM, pseudopods presented a globularlike structure at the extremity, similar to a dense nest located immediately below the epidermal basal layer and characterized by sharp borders only in the outside front, but connected at the lesion core by a sheet of loosely aggregated cells, giving rise to a comet star–like appearance. On histologic analysis, a well-defined nest, located at the tip of the enlarged and parallel oriented crista, was observable (Figure 13).

Diffuse pigmentation was studied in 95 lesions, belonging to 36 nevi and showing a light brown pigmentation, 33 lesions (17 MMs, 11 nevi, and 5 Spitz or Reed nevi) with dark brown to black homogeneous diffuse pigmentation, and 26 lesions (19 MMs and 7 nevi) with
dark pigment blotches. Light brown homogeneous pigmentation was characterized in superficial layers by a honeycombed pattern. Immediately below this area, a subtle network of small regular edged papillae, occasionally alternated with small, weakly reflecting, dense, regular nests, was observed in all cases. Histologic analysis revealed little pigmentation in the epidermal layers (Figure 14).

On RCM, dark diffuse pigmentation and pigment blotches showed a high reflectivity in the superficial layers owing to the abundant content of melanin within the keratinocytes, resulting in a bright cobblestone pattern (Figure 15). In 21 of 36 MMs and 2 of 5 Spitz or Reed nevi, the RCM cobblestone pattern was intermingled with large pagetoid cells (Figure 16). Immediately below the superficial layers, 31 MMs, 2 nevi, and 3 Spitz or Reed nevi showed a disarrangement of the dermoepidermal architecture and/or single or clustered atypical cells, whereas regular edged papillae and dense regular nests were usually observable in benign lesions. Plump bright cells were also present within the dermal papillae in some benign and malignant lesions. The brightness of the keratinocytes corresponded to abundant pigmentation within keratinocytes on histologic examination. Confocal pagetoid infiltration and cytologic and architectural atypia were confirmed by histopathologic analysis.

Figure 13. Histologic analysis revealed discrete nests at the edge of the lesion. Pseudopods in a nevus as shown by dermoscopy (white dotted square corresponds to the reflectance confocal microscopy [RCM] image) (A) (original magnification ×50) that correspond to dense nests bridged with the lesion core by a sheet of loosely aggregated cells, giving rise to a comet like appearance on RCM (white arrow) (B) (original magnification ×30). Histologic analysis revealed a well-defined nest, located at the tip of the enlarged and parallel oriented crista (white arrowhead) (white dotted line corresponds to the level of the RCM image) (C) (hematoxylin-eosin, original magnification ×200).

Figure 14. Histologic analysis revealed little pigmentation in the epidermal layers. Light brown homogeneous pigmentation of a nevus as observable by dermoscopy (white dotted square corresponds to the reflectance confocal microscopy [RCM] image) (A) (original magnification ×50) was characterized by a honeycombed pattern in superficial layers on RCM (B) (original magnification ×30) and corresponded to scarcely pigmented epidermal layers on histologic analysis (white dotted line corresponds to the level of the RCM image) (C) (hematoxylin-eosin, original magnification ×200).

Figure 15. Histologic analysis revealed abundant transepidermal melanin dispersion. A, Dark diffuse pigmentation in a nevus on dermoscopy (white dotted square corresponds to the reflectance confocal microscopy [RCM] image) (original magnification ×30). The RCM revealed high reflective superficial layers giving rise to a bright cobblestone pattern (B) (original magnification ×30) caused by the abundant content of melanin within the keratinocytes (white dotted line corresponds to the level of the RCM image) (C) (hematoxylin-eosin, original magnification ×200).
Blue structures were observed in 32 cases, including 20 MMs, 1 nevus, and 1 Spitz or Reed nevus with a blue-whitish veil and 3 MMs, 5 nevi, and 2 Spitz or Reed nevi with blue areas. A homogeneous steel blue pigmentation was present in 5 blue nevi.

All MMs and the Spitz or Reed nevus characterized by the blue veil showed epidermal disarray, roundish pagetoid infiltration, nonedged papillae, and cytologic atypia in the basal layer. Dishomogeneous nests were preset in 12 lesions and cerebriform clusters in 8, along with nu-

Figure 16. Histologic analysis revealed pigmented keratinocytes and numerous pagetoid cells in the epidermis. Dark diffuse pigmentation in a melanoma on dermoscopy (white dotted square corresponds to the reflectance confocal microscopy [RCM] image) (A) (original magnification ×20). The RCM showed a cobblestone pattern intermingled with large round to oval cells with bright cytoplasm and dark nucleus (white arrows) (B) (hematoxylin-eosin, original magnification ×30) that correspond to pagetoid infiltration on histologic analysis (white arrowheads) (white dotted line corresponds to the level of the RCM image) (C) (hematoxylin-eosin, original magnification ×100).

Figure 17. Histologic analysis revealed epidermal disarray and pagetoid infiltration, and nondiscrete nests, malignant melanocytes, and marked inflammatory infiltrate in the dermis. A, Blue veil in a melanoma on dermoscopy (white dotted square corresponds to the reflectance confocal microscopy [RCM] image) (original magnification ×20). B, Histologic analysis showed parakeratosis, pagetoid infiltration, and architectural disarray with marked cytologic atypia (white arrowheads) (white dotted line corresponds to the level of the RCM image) (original magnification ×200). The RCM showed a disarranged pattern and roundish pagetoid infiltration in the epidermis (white arrows) (C) (original magnification ×30) and nonedged papillae, atypical cells in the basal layer (white arrow) and in the upper dermis (white asterisk), and melanophages (white arrowhead) (D) (hematoxylin-eosin, original magnification ×30).
merous nonaggregated cells infiltrating the dermal papillae, corresponding to a collection of plump bright and nucleated cells (Figure 17). On histopathologic analysis, epidermal alterations corresponded to orthokeratosis and parakeratosis associated with marked pagetoid melanocytosis. The dermoeipidermal contour disappeared completely, presenting marked cytologic atypia. Nests of malignant cells crowded against the epidermis and infiltrated the dermis, along with cords and single cells deeply invading the dermis and intermingling with a marked inflammatory infiltrate and melanophages. On the other hand, the nevus with a blue veil on dermoscopy revealed a thickened epidermis and abundant melanophages in the dermis on RCM and histologic analysis.

Exploring blue areas by means of RCM, the areas were found to correlate with the presence of plump bright cells, corresponding to melanophages on histologic analysis (Figure 18), in all nevi but 1, which showed dense clusters in depth. Isolated nucleated cells intermingled with plump bright ones were present within dermal papillae in all MMMs and correlated with infiltrating malignant melanocytes. Moreover, some RCM features suggesting an MM diagnosis, such as a disarranged epidermal pattern, roundish pagetoid cells, nonedged papillae, and cytologic atypia, as well as their histopathologic substrates, were identified in all malignant lesions and in 2 Spitz or Reed nevi, whereas they were absent in common nevi.

In the 5 blue nevi, a normal honeycombed pattern and a regular pattern composed of slight reflecting rings of cells surrounding dark empty papillae, not different from healthy skin, were observable on RCM. Histopathologic analysis showed ill-defined deep dermal proliferation of elongated or dendritic melanocytes. The depth of the localization of the cellular component in blue nevi seemed to be responsible for the lack of RCM findings.

Regression was evaluated in 34 MMMs and in 3 nevi; some of the cases were characterized by extensive regression areas with blue pepperlike granules. All cases showed few epidermal layers characterized by a honeycombed pattern; only 7 MMMs also showed a few pagetoid cells. The dermoeipidermal boundary was imperceptible, passing directly from the honeycombed epidermal layers to the dermis without the appearance of the reflective basal cells and papillary contours. The dermis consisted of a coarse network of ill-defined grainy bundles or, less frequently, of fibers oriented in the same direction. Small bright reflecting spots and plump bright cells, intermingled with collagen bundles, were usually observable in correspondence where the peppering is shown. Few nucleated cells with bright cytoplasm and well-defined borders, suggestive of malignant melanocytes infiltrating the dermis, were present in only 4 MMMs (Figure 19). Histopathologic analysis revealed a thin, atrophic epidermis devoid of melanin, covering areas of fibroplasia. Inflammatory infiltrate, consisting of leukocytes and a few melanophages, was present within fibroplasia. Also on histologic analysis, malignant cells were seldom observed within the regression area.
Dermoscopy used by experts yielded an improvement in diagnostic accuracy, in particular for thin MMs. However, to rule out malignant neoplasms with high sensitivity, numerous nevi are excised owing to dermoscopic atypical features akin to MMs. Recently, the introduction of RCM in the field of skin oncology showed the capability to produce real-time in vivo sections of the skin at a nearly histologic resolution, enabling the clear and well-contrasted visualization of cells and structures at a maximum depth of approximately 250 µm. The histologic correspondence of some confocal features has been demonstrated, although numerous patterns still have to be clearly defined. The increasing interest in using RCM in specialized skin cancer centers derives from the possibility of having a more accurate presurgical diagnosis for different skin tumors, resulting in demonstrated improvement in diagnostic accuracy, especially for basal cell carcinoma and melanocytic lesions, also with respect to dermoscopy. The need to position a metal ring with adhesive tape for the examination of a single lesion limits RCM’s application as a screening tool, and its use remains restricted to selected lesions. For this reason, RCM can be efficiently applied on dermoscopically difficult lesions, enabling the improvement of diagnostic specificity. Recently, Scope and coworkers demonstrated the good positive correlation between the global dermoscopic pattern and confocal mosaics at the dermoepidermal junction and a correspondence between confocal aspects and specific dermoscopic features, such as atypical pigment network, pigment globules, peripheral streaks, and a blue-whitish veil, on 11 lesions. Subsequently, the same authors focused their analysis on 7 cases that presented with peripheral streaks, enabling confocal-histologic relations and distinction among different morphologic subtypes on the basis of their confocal aspects.

This study aimed to explore systematically the confocal substrates of the dermoscopic features of melanocytic lesions, with the exception of site-specific patterns, to obtain a comprehensive description and characterization of the underlying cytologic and architectural features and to correlate them with histopathologic features. In detail, the identification of single large, bright cells in superficial layers, the basal layer, and the papillary dermis or the presence of clusters of pleomorphic cells may help to interpret some underlying dermoscopic features that are characteristic of malignant neoplasms. The overall analysis of RCM features in a large population of dermoscopically equivocal melanocytic lesions showed the capability of this technique in improving diagnostic specificity for MM. On the other hand, the present study suggests that RCM examination should be conducted focusing on specific dermoscopic features to better discover RCM diagnostic clues, whereas areas presenting with dermoscopic features that usually lack RCM diagnostic substrates should be avoided. Tight correspondence between network holes and dermal papillae, network meshes and interpapillary spaces, and pigment globules and cell clusters was confirmed. Cyto-logic and architectural atypia on RCM was predominantly present in MMs and strongly correlated with histologic features. Otherwise, the observation of large cells within dense clusters in dermal nevi should not be interpreted as cytologic atypia, corresponding to larger melanocytes in the upper portion of the dermal nests or cords. Although different RCM substrates were confirmed for peripheral streaks, MM-specific aspects were not observable within these structures. Moreover, the histologic substrate of pigment dots was clearly identifiable by RCM, distinguishing between pagetoid melanocytosis, suggestive of a malignant neoplasm, and melanin clumps within the epidermis.

The presence of confocal features suggestive of malignancy, such as atypical melanocytes, pagetoid cells, or nonedged papillae, turned out to be useful for the correct interpretation of dermoscopically indeterminate aspects, such as dark pigmentation, pigment blotches, or blue structures. As recently demonstrated, RCM was particularly useful for the interpretation of the bluish pigmentation, enabling the distinction between inflammatory infiltrate, predominantly constituted by plump bright cells within dermal papillae corresponding to mela-nophages, and malignant melanocytic cells, which singularly or in clusters infiltrate the dermis in invasive MMs. On the other hand, within regression areas, RCM failed to identify MM-specific aspects in most cases.

In conclusion, the knowledge of the cytologic and architectural aspects of the different dermoscopic patterns, as they appear by means of RCM, may be useful for the detection of specific substrates in MMs and may lead to a more accurate interpretation of the dermoscopic alterations. Furthermore, the possibility of recognizing in vivo cytologic patterns and following them up over time may help to identify MM precursors and to understand the biology of melanocytic lesions.

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