Reflectance-mode confocal microscopy of pigmented skin lesions–improvement in melanoma diagnostic specificity

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Background: In vivo confocal microscopy enables skin visualization with a quasihistopathologic resolution.

Objective: We sought to describe confocal features in melanocytic lesions and to evaluate their diagnostic significance for melanoma (MM) identification.

Methods: Thirty seven MMs, 49 acquired nevi, and 16 Spitz/Reed nevi, presenting equivocal clinicodermoscopic aspects were investigated by confocal microscopy.

Results: MMs and nevi significantly differed for some aspects. In multivariate analysis, the presence of nonedged dermal papillae, atypical cells, and isolated nucleated cells within dermal papilla, pagetoid cells, widespread pagetoid infiltration, and cerebriform clusters were strongly correlated with MM diagnosis. A receiver operating characteristic curve value of 0.952 was obtained.

Limitations: Spitz/Reed nevi represented a pitfall in confocal diagnosis, owing to the frequent observation of pagetoid infiltration, architectural disarray, and cytologic atypia, and to the impossibility of evaluating cell maturation with depth.

Conclusion: Characterization of confocal microscopy features of MMs and nevi seems to improve diagnostic accuracy for melanocytic lesions that are difficult to diagnose. (J Am Acad Dermatol 2005;53:979-85.)

Increase in incidence and mortality for melanoma (MM) is observed worldwide.1 Because adequate therapies are lacking for the advanced disease, the best treatment is represented by early diagnosis and prompt surgical excision of the tumor. In the past 2 decades the worldwide diffusion of dermoscopy, a noninvasive low-cost diagnostic technique that enables the visualization of subsurface structures, improved MM diagnostic accuracy.2,3 The recent introduction of in vivo reflectance-mode confocal laser microscopy (RCM), enabling the instant visualization of skin structures at a quasihistopathologic resolution, represents a new noninvasive approach for the in vivo study of physiologic and pathologic conditions of the skin.4,5 Because melanin and melanosomes are strong sources of contrast, melanocytic cells are particularly evident by means of this technique.4,6 In preliminary studies, some RCM features of melanocytic lesions have been identified,7-10 suggesting the possibility of a further improvement in diagnostic accuracy of MM,11 especially when this technique is used in combination with dermoscopy.12-14 The aim of our study was to evaluate the frequency of confocal features in benign and malignant melanocytic lesions and their diagnostic significance for MM identification. A second end point was to identify the most relevant features for MM diagnosis and to combine them in a simple algorithm useful for practical diagnostic purposes.

METHODS

Images

This study included a total of 16,618 images acquired by means of RCM referring to 102...
consecutive melanocytic lesions, 5906 of which referred to 37 MMs, 7693 to 49 acquired nevi (21 junctional, 27 compound, and 1 intradermal), and 3019 to 16 epithelioid and/or spindle cell nevi (3 junctional Spitz, 8 compound Spitz, and 5 Reed), corresponding to an average of 163 images/lesion. All malignant lesions were superficial spreading MMs (18 cases on the trunk and 19 on the limbs) with a mean Breslow thickness of 0.8 ± 0.77 mm. In 4 cases they were in situ, 24 lesions corresponded to MMs thinner than 1 mm (pathologic stage T1 in accordance with American Joint Committee on Cancer classification15), 5 lesions were 1.01- to 2.0-mm thick (pathologic stage T2), and 4 were 2.01- to 4.0-mm thick (pathologic stage T3). Nodular MMs were not included in the study. For all benign lesions, excision was performed to rule out MM. Benign lesions presented equivocal aspects at clinical and/or dermoscopic inspection, with a mean score, calculated according to the new 7-point checklist,16 of 3.88 (SD 1.63) for melanocytic nevi and 4.12 (SD 1.67) for Spitz/Reed nevi. Six melanocytic nevi with a new 7-point check list score lower than 3 (corresponding to the threshold for malignancy) were characterized by a history of a recent modification. Lesions of the face, scalp, palm, and soles were not included in the study.

Instruments and acquisition procedure
Before biopsy, RCM images were acquired by means of a near-infrared reflectance confocal laser scanning microscope (Vivascope 1000, Lucid Inc, Henrietta, NY), which uses an 830-nm laser beam with a maximum power of 35 mW. Instrument and acquisition procedures are described elsewhere.4 Each image corresponds to a horizontal section at a selected depth with an effective 475- × 350-μm field of view, and a resolution of 640 × 480 pixels and 255 colors (spatial resolution of 1.0 μm in the lateral dimension and 4.5 μm in the axial dimension). A sequence of 30 montage images (block image) was acquired for each lesion at superficial layer, dermoepidermal junction, and papillary dermis level to explore a 7.60- × 6.65-mm field of view/lesion.12 Moreover, confocal sections, beginning at the stratum corneum and ending inside the papillary dermis, were recorded at areas of interest.

RCM feature description

Superficial (granulosum/spinosum) layers.

General aspect. The superficial layer was evaluated identifying 3 possible patterns: honeycombed pattern, formed by 10- to 20-μm polygonal cells with dark nuclei and bright and thin cytoplasm; cobblestone pattern, consisting of small polygonal cells with refractive cytoplasm separated by a less refractive border; and disarranged pattern, characterized by disarray of the normal architecture of the superficial layers with unevenly distributed bright granular particles and cells.7

Presence and aspects of pagetoid cells. Pagetoid cells were considered when large nucleated cells with refractive cytoplasm and dark nucleus were observable within superficial layers.8-10 We reported pagetoid cell density, regarded as numerous or sporadic cells; cell shape, evaluated as roundish or dendritic; cell pleomorphism; cell distribution, referred to as focal or widely diffused; and extension to the stratum corneum, when cells were observable up to 20 μm below the surface.10

Basal cell layer and dermopidermal junction. By means of RCM, basal cells appear at a depth of approximately 50 to 100 μm below the stratum corneum. Going in depth, dermal papillae correspond to dark round to oval areas circumscribed by refractive cells, corresponding to melanocytes and melanin-rich keratinocytes.

Dermal papilla features.14 Dermal papillae circumscribed by a rim of refractive cells, appearing as bright rings sharply contrasting with the dark background, were defined as edged papillae, in contrast with the observation of dermal papillae without a demarcated rim of bright cells, but separated by a series of large reflecting cells, defined as nonedged papillae.

Cellular clusters within basal layer. The presence of clusters of reflecting cells corresponding to junctional melanocytic nests was described. Oval compact cellular aggregates bulging within the dermal papilla, directly in connection with the basal cell layer, were called junctional clusters, whereas enlargements of the intrapapillary space formed by aggregated cells were defined as junctional thickenings.

Cellular aspects.14 Basal layer cells were considered large when a mean cell area greater than 250 μm² (corresponding to a mean diameter of approximately 18 μm) was measured. Cells were morphologically described considering cytologic atypia throughout the lesion. Three different degrees of cellular atypia were identified: monomorphous small cells were defined as typical; mild atypia was considered when irregular larger cells were sporadically observable within typical cell architecture; and marked atypia was defined as numerous cells irregular in size, shape, and reflectivity, round to oval or stellate, occasionally with branching dendritic-like structures, distributed throughout the lesion. Moreover, the presence of stretches of numerous highly reflective cells not interrupted by dermal papillae was also reported.
Papillary dermis.

Melanocytic nest features. The presence of clusters of refractive cells forming oval to roundish structures immediately below the basal cell layer, corresponding to melanocytic nests, was reported. According to their aspect, cellular clusters were divided into 3 different types. Compact aggregates of large polygonal cells were defined as dense clusters, whereas roundish nonreflecting structures with a well-demarcated border, containing bright nucleated cells, were defined as sparse cell clusters. Cellular clusters consisting of confluent aggregates of low reflecting cells, brainlike in appearance, were named cerebriform clusters.13

Isolated cells within papillary dermis. The presence of nonaggregated cells inside dermal papillae was reported distinguishing between round to oval cells with refractive cytoplasm and eccentric dark nucleus, corresponding to melanocytes infiltrating dermal papilla, and plump irregularly shaped bright cells with ill-defined borders and no visible nucleus, corresponding to melanophages.6,7

Statistics

Statistical evaluation was carried out using software (statistical package, release 10.0.6, SPSS Inc, Chicago, Ill). Statistical analysis was performed on data referring to all the examined lesions for epidermal features and the presence of plump bright cells within the papillary dermis, and on data referring to lesions with dermal component (invasive MMs and compound or intradermal nevi) for melanocytic nest features and the presence of nucleated cells within dermal papilla. Absolute and relative frequencies of each confocal criterion were calculated in MMs, melanocytic nevi, and Spitz/Reed nevi. Significant differences between MMs and benign lesions (melanocytic nevi + Spitz/Reed nevi) were evaluated by means of the Chi-square test of independence (Fisher’s exact test was applied if any expected cell value in the 2 × 2 table was <5). For an estimate of MM risk, a calculation of the odds ratio and 95% confidence interval was performed. Using the discriminant analysis approach, RCM features useful for the distinction between benign and malignant melanocytic lesions were identified and the individual criteria were scored 1 or 2 points according to the Chi-square value inferior or greater than 20, respectively. The leave-one-out method was used to evaluate the predictive performance of the classification rules step by step. This allowed the creation of a simple diagnostic method (suitable for clinical use) based on identification of major and minor RCM criteria for MM diagnosis. A total score was obtained for each lesion. Receiver operating characteristic analysis was performed to investigate sensitivity and specificity of this method. The area under the curve, which represents an index of the overall discriminant power, was calculated by the nonparametric trapezoidal method. Sensitivity, specificity, diagnostic accuracy, odds ratio, and 95% confidence interval were calculated for each score value. A P value less than .05 was considered significant.

RESULTS

RCM features

Superficial (granulosum/spinosum) layers. RCM aspects of stratum granulosum/spinosum are summarized in Table I. Melanocytic lesions were usually characterized by honeycombed pattern, cobblestone pattern, or both. The presence of a disarranged table.

<table>
<thead>
<tr>
<th>General aspect of superficial layers:</th>
<th>MM (37)</th>
<th>Nevi (49)</th>
<th>Spitz/Reed (16)</th>
<th>Total (102)</th>
<th>OR (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Honeycombed</td>
<td>17* (45.9%)</td>
<td>36 (73.5%)</td>
<td>12 (75%)</td>
<td>65 (63.7%)</td>
<td>0.30 (0.13-0.71)</td>
</tr>
<tr>
<td>Cobblestone</td>
<td>14 (37.8%)</td>
<td>24 (49%)</td>
<td>6 (37.5%)</td>
<td>44 (43.1%)</td>
<td>NS</td>
</tr>
<tr>
<td>Disarranged</td>
<td>15* (40.5%)</td>
<td>0 (0%)</td>
<td>1 (6.3%)</td>
<td>16 (15.7%)</td>
<td>43.6 (5.4-349.8)</td>
</tr>
</tbody>
</table>

Pagetoid infiltration:

<table>
<thead>
<tr>
<th>Presence of pagetoid cells</th>
<th>MM (37)</th>
<th>Nevi (49)</th>
<th>Spitz/Reed (16)</th>
<th>Total (102)</th>
<th>OR (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numerous cells</td>
<td>16* (43.2%)</td>
<td>0 (0%)</td>
<td>1 (6.3%)</td>
<td>17 (16.7%)</td>
<td>48.8 (6.1-390.1)</td>
</tr>
<tr>
<td>Roundish cells</td>
<td>31* (83.8%)</td>
<td>2 (4.1%)</td>
<td>2 (12.5%)</td>
<td>35 (34.3%)</td>
<td>78.8 (20.7-300)</td>
</tr>
<tr>
<td>Dendritic cells</td>
<td>18* (48.6%)</td>
<td>2 (4.1%)</td>
<td>4 (25%)</td>
<td>24 (23.5%)</td>
<td>9.32 (3.2-26.8)</td>
</tr>
<tr>
<td>Pleomorphism</td>
<td>27* (73%)</td>
<td>2 (4.1%)</td>
<td>2 (12.5%)</td>
<td>31 (30.4%)</td>
<td>41.2 (11.9-142.9)</td>
</tr>
<tr>
<td>Widely diffused cells</td>
<td>21* (56.8%)</td>
<td>0 (0%)</td>
<td>1 (6.3%)</td>
<td>22 (21.6%)</td>
<td>84.0 (10.5-672.1)</td>
</tr>
<tr>
<td>Extension to the stratum corneum</td>
<td>26* (70.3%)</td>
<td>2 (4.1%)</td>
<td>2 (12.5%)</td>
<td>30 (29.4%)</td>
<td>36.1 (10.5-123.7)</td>
</tr>
</tbody>
</table>

CI, Confidence interval; MM, melanoma; NS, not significant; OR, odds ratio.

*Significant (P < .05) compared with benign lesions (acquired nevi + Spitz nevi).
pattern was specific for MM diagnosis, because it was observed in 40% of malignant lesions and in only one Spitz nevus. Pagetoid cells were observed in the majority of MMs and in less than 11% of benign lesions, especially in Spitz nevi (4/11 cases). Numerous cells were observed in approximately 50% of MMs with pagetoid melanocytosis, whereas the majority of benign lesions presented a slight to medium cellular density. Pagetoid cells were frequently roundish, although sometimes dendritic cells were observable, especially in benign lesions. The widespread distribution and extension to the stratum corneum of pagetoid cells was significantly correlated with malignancy.

**Basal cell layer and dermoepidermal junction.** Dermal papilla features, junctional nests, and cellular aspects at basal layer are described in Table II. The majority of acquired nevi were characterized by edged papillae, in contrast with the observation of nonedged papillae in approximately 95% of MMs and 75% of Spitz/Reed nevi.

At the dermoepidermal junction, directly connected to the basal cells, junctional clusters were observed in 42.2% of lesions, whereas junctional thickenings were reported in 44.1% of cases, without significant differences between benign and malignant lesions. In the remaining 13 cases, with histopathologic junctional component, clusters at dermoepidermal layers were not clearly distinguishable.

At basal layer, MMs were characterized by larger and more irregular cells (corresponding to cytologic atypia) compared with nevi. The presence of marked cytologic atypia throughout the lesion, reported in 56% of MMs and in less than 5% of benign lesions, appeared as a specific marker of malignancy. Moreover, in approximately 65% of MMs and only in two benign lesions the confocal reticular architecture, represented by lines of cells and dermal papillae, was occasionally interrupted by stretches of numerous irregularly shaped highly reflective cells.

**Papillary dermis.** Melanocytic clusters were observed in approximately 65% of lesions, exactly...
corresponding to invasive MMs and compound melanocytic and Spitz/Reed nevi, with the exception of one Spitz and one melanocytic nevus, where clusters were not clearly identifiable and dermal component was constituted by isolated cells (Table III). In the majority of cases the dense cluster type was observed. The sparse cell cluster type was predominantly observed in MMs, but also reported in one acquired and one Reed nevus. Cerebriform clusters were solely present in MMs, but only in 7 of 33 cases.

Isolated cells with eccentric dark nucleus and bright cytoplasm localized within dermal papilla, corresponding to melanocytes, were considered a specific marker of malignancy, being present in 45.5% of invasive MMs and only in two benign lesions (Table III).

On the other hand, plump bright cells with no evident nuclei, corresponding to melanophages, had no diagnostic value, because they were present in 18 of 37 MMs (48.6%), 18 of 49 melanocytic nevi (36.7%), and in 8 of 16 Spitz/Reed nevi (50%).

**Multivariate analysis**

By means of discriminant analysis, 6 criteria were identified as independently correlated with a MM diagnosis. The two major criteria, characterized by a Chi-square value greater than 20 and scored 2 points in the diagnostic algorithm, corresponded to the presence of cytologic atypia (mild or marked) and of nonedged papillae at basal layer (Fig 1). The 4 minor criteria, characterized by a Chi-square value inferior to 20 and scored 1 point in the diagnostic algorithm, were represented by the presence of roundish cells in superficial layers spreading upward in a pagetoid fashion, pagetoid cells widespread throughout the lesion, cerebriform clusters in the papillary dermis, and nucleated cells within dermal papilla (Fig 2).

A total score, ranging from 0 to 8, was calculated for each lesion and a receiver operating characteristic curve with an area under the curve of 0.952 (95% confidence interval = 0.905-1.000) was obtained. Considering lesions with a score equal to or greater than 3, a 97.3% sensitivity and a 72.3% specificity (82.6% for melanocytic nevi and 43.8% for Spitz/Reed nevi) were obtained, whereas increasing the threshold to a score equal to or greater than 5, specificity increases to 96.9%, with a decrement of sensitivity to 83.8%. A 100% specificity with 37.8% sensitivity was reached with scores equal to or greater than 7.

**DISCUSSION**

MM incidence has dramatically increased in past years. Because survival is strongly dependent on tumor thickness, early diagnosis is essential for patient survival. Dermoscopy has been demonstrated to improve diagnostic accuracy, especially for thin MMs. Although the use of dermoscopy in routine MM screening is followed by an improvement of the malignant/benign ratio in excised lesions, more than 5 lesions have to be removed to find one MM, owing to the impossibility to distinguish between nevi with dermoscopic atypia and thin MMs. RCM offers the possibility to noninvasively investigate the cytologic and architectural aspects of clinically difficult lesions, evidencing features reported as suggestive of MM diagnosis and substrates of characteristic dermoscopic patterns, such as pigment network and globules. Because light source limits the depth of penetration to approximately 200 to 300 μm, permitting the characterization of superficially located structures and cells, this technique appears particularly suitable for differentiation between flat to palpable pigmented lesions with atypical features at dermoscopic...
observation. Some features have been previously identified as indicators of MM diagnosis. Exploring 6 MMs and 34 nevi, the disarrangement of the normal honeycombed architecture of superficial layers and the presence of individual cells, polymorphic in size and shape, sometimes spreading upward in a pagetoid fashion, were reported as specific MM markers. Moreover, Busam et al found the presence of pagetoid cells and of enlarged (atypical) melanocytes in 5 thin MMs. Case reports on one lentigo maligna MM and two amelanotic MMs showed the usefulness of RCM for diagnostic confirmation and surgical margin assessment. Very high sensitivity and specificity values of some confocal features, such as melanocyte cytology, disarray of the architecture, and poorly defined keratinocyte cell borders, were identified by Gerger et al comparing two preselected images per lesion from a database of 27 MMs and 90 nevi, the majority of which corresponded to clearly benign lesions.

The aim of this study was to evaluate the diagnostic significance of RCM features for differentiating between benign and malignant melanocytic lesions. We investigated a large series of lesions characterized by the presence of clinical and dermoscopic features suggestive of MM diagnosis. An area of approximately 7 mm² was explored in each lesion at 3 different depth levels, superficial layers, dermoepidermal junction, and papillary dermis, evaluating approximately 163 images/lesion. The presence and the aspects of some RCM features were described in blind. Benign and malignant lesions significantly differed for the presence of some features, such as pagetoid infiltration, disarray of dermoepidermal junction architecture, atypical melanocytic cells within the basal layer and inside the papillary dermis, and sparse cell and cerebriform nests within papillary dermis. Moreover, we designed a diagnostic model that requires the identification of only 6 features, independently correlated with MM diagnosis. We considered the presence of at least two features, one major and one minor criterion (score ≥ 3) essential for MM diagnosis. Using this algorithm all MMs except one and 47 of 65 benign lesions were correctly diagnosed. The only misclassified MM did not present diagnostic criteria at RCM and just a slight pattern asymmetry and a focally broadened network at dermoscopic inspection together with an anamnestic report of recent modification. Histopathologic
diagnosis derived from the observation of a focus of atypical cells inside a common nevus. Because the lesion measured approximately 12 × 7 mm, we assumed that the area of interest was out of the area explored by means of RCM. Spitz/Reed nevi represented a pitfall in RCM diagnosis: in fact 9 of 16 cases were misclassified, owing to the frequent observation of pagetoid infiltration in superficial layers, architectural disarray and cytologic atypia at basal layers, and nucleated cells within dermal papillae, and to the impossibility of evaluating essential diagnostic criteria, such as cell maturation with depth. Increasing the algorithm threshold to 7, corresponding to the presence of at least 5 of 6 criteria, 100% specificity was achieved, enabling the possibility of an adequate surgical approach before histopathologic confirmation.

In conclusion, RCM was useful for the in vivo characterization of equivocal melanocytic lesions at clinical and dermoscopic evaluation. Although RCM examination is still time-consuming, especially for the systematic exploration of a wide portion of the lesion area at 3 depth levels, the algorithm may facilitate the practical application of this device and improve diagnostic specificity of melanocytic lesions when applied on difficult cases.

REFERENCES