In Vivo Microscopic Features of Nodular Melanomas

Dermoscopy, Confocal Microscopy, and Histopathologic Correlates

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Objective: To characterize nodular melanoma (NM) using dermoscopy, in vivo reflectance-mode confocal microscopy, and histopathologic analysis.

Design: Consecutive pure NMs and superficial spreading melanomas (SSMs) with nodular or blue areas were studied using dermoscopy and confocal microscopy, and a correlation with histopathologic findings was performed.

Materials: Ten NMs, 10 SSMs with a nodular area, and 10 SSMs with a blue palpable but not yet nodular area.

Main Outcome Measure: Confocal differences within the nodular component between pure NMs and SSMs with a nodular area, hypothesizing different biological behaviors.

Results: Whereas NMs had predominantly nonspecific global dermoscopic patterns, SSMs exhibited a multicomponent pattern and higher dermoscopic scores. Globules, blue-white veil, atypical vessels, and structureless areas were frequent in NMs and in nodular areas from SSMs. At confocal microscopy, NMs exhibited few pagetoid cells within a typical epidermal architecture in the superficial layers in most cases, differing from SSMs frequently characterized by epidermal disarrangement and pagetoid infiltration. At the dermoepidermal junction, dermal papillae were rarely seen in nodular areas both from NMs and from SSMs, frequently substituted by nonaggregated atypical cells distributed in sheetlike structures. In the upper dermis, all groups exhibited plump bright cells, dense dishomogeneous cell clusters, and atypical nucleated cells, whereas cerebriform clusters were characteristic of NMs.

Conclusion: Distinctive dermoscopic and confocal features seen in NMs compared with SSMs are helpful in making the diagnosis and suggest different biological behavior.

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Nodular melanoma (NM) is responsible for approximately 9% to 15% of invasive melanomas and as many as 50% of melanomas thicker than 2 mm. Nodular melanoma arises in healthy skin or in a precursor lesion but without the presence of a radial growth phase. Thus, even in its early stages, NM has the potential to metastasize. The classification of melanoma by subtype is based on anatomical and epidemiological features and pattern of progression. However, recent research has shown molecular and genetic differences between melanoma subtypes. These differences may explain the difference in the natural evolution of melanoma subtypes. In NM, classic clinical criteria for diagnosis of melanoma, with the exception of change, fail because these tumors are often small, round, and symmetric, with regular borders.

The color is often homogeneous compared with that of superficial spreading melanoma (SSM) and may be pink or red rather than black, blue, or brown. In some cases, NM can be hypochromic or amelanotic. Because of these peculiarities, diagnosis of NM is challenging, and misdiagnosis at the first consultation leads to delay in treatment and worse prognosis.

Dermoscopy of NM is also difficult because the asymmetric pattern is less marked than in SSM. Nevertheless, irregularity in color is usually present in pigmented NMs. In NM, many of the classic dermoscopic features of SSM are usually lacking, especially those dermoscopic structures that correspond to the flat parts of the SSM. Pigment network is often absent in NM, with the ex-
To date, features of RCM for nodular melanoma have not been described, and studies of dermoscopic criteria for these lesions are also lacking. To determine confocal, dermoscopic, and histologic features in NM, we examined 10 NMs and compared them with 10 SSMs with a nodular area (SSM$_{nod}$) and 10 SSMs with a blue palpable area not yet nodular (SSM$_{blue}$).

### METHODS

**PATIENTS**

Thirty patients were recruited from the Departments of Dermatology of 3 medical centers: Hospital Clinic, Barcelona, Spain; University of Modena and Reggio Emilia, Modena and Reggio Emilia, Italy; and Royal Prince Alfred Hospital, University of Sydney, Sydney, Australia. Patients gave informed consent for RCM examination of lesions. The dermoscopic evaluation was performed by 2 clinicians (S. Segura and J.M.), as were the confocal evaluation (S. Segura and G.P., who were not blinded to the dermoscopic images) and the histologic evaluation (S. Segura and J.P.). The agreement between RCM and histopathologic findings was made by a single nonindependent observer (S. Segura).

### REFLECTANCE-MODE CONFOCAL MICROSCOPY

Confocal imaging was performed with near-infrared reflectance-mode confocal laser scanning microscopes (Vivascope 1000 and Vivascoppe 1500; Lucid Inc, Rochester, New York). The instruments use a diode laser at 830 nm with a power of less than 16 mW at tissue level and ×30 water-immersion lenses enabling a horizontal optical resolution of 2 µm and a vertical resolution of 5 µm. Instruments and acquisition procedures have been described elsewhere. Each image corresponded to a horizontal section at a selected depth with an effective field of view of 475 × 350 µm for the Vivascope 1000 and 500 × 500 µm for the Vivascoppe 1500. Block images were acquired for each lesion to explore a 4 × 4-mm field of view. Confocal sections and vertical montage images (stack images) from the stratum corneum to the papillary dermis were recorded at areas of interest. Already described confocal criteria for benign and malignant melanocytic lesions were systematically evaluated.

### DERMOSCOPIC STUDY

The lesions were evaluated and documented by epiluminescence microscopy using different devices in the 3 study sites: a commercially available videodermoscope (FotoFinder;...
TeachScreen Software GmbH, Bad Birnbach, Germany), a dermoscope (DermLite DL100; 3 Gen LLC, San Juan Capistrano, California) in combination with a digital camera for dermoscopic photographs (DermLite FOTO; 3 Gen LLC), and a high-resolution digital oil immersion dermoscopy camera (SolarScan Sentry; Polartecnics Ltd, Sydney, Australia). Spatial orientation of lesions for dermoscopy and confocal correlation was performed using an external macrocamera (VivaCam; Lucid Inc) adapted to the confocal microscope, which was available only in the centers equipped with the Vivascope 1500 scanning microscope.

HISTOPATHOLOGIC STUDY

After tumor excision, the tissue was fixed in 10% formalin and embedded in paraffin. After routine processing, the slides were stained with hematoxylin-eosin. For confocal, dermoscopic, and histopathologic correlations, sections passing through the nodule in NM and SSMNod were obtained from each lesion. In the SSMBlue group, the study area was where the tumor was thicker because that is the part that corresponds to the clinically palpable area.

STATISTICAL ANALYSIS

Statistical evaluation was carried out using the SPSS statistical software package for Windows (version 11.0; SPSS, Inc, Chicago, Illinois) and performed on data referring to all of the lesions for confocal, dermoscopic, and histopathologic features. Absolute and relative frequencies of each confocal, dermoscopic, and histopathologic criterion were evaluated in the NM, SSMNod and SSMBlue groups. Significant differences between NM and SSMNod and between both SMM groups were evaluated using the χ² test of independence (the Fisher exact test was used if any expected cell value was <5 in the 2×2 table). For the confocal, dermoscopic, and histopathologic correlations, the Cohen κ index was calculated for each descriptor. κ Values ranged between 1 and 0. A κ value of 1.00 indicates full agreement beyond chance, values greater than 0.70 are generally considered excellent, values less than 0.40 are considered poor, and values between 0.40 and 0.70 are considered fair to good.

RESULTS

GENERAL OBSERVATIONS

Demographic data for the study population and Breslow thickness of the tumors are given in Table 1. Clinically, NM were small to medium, measuring 10 mm or less in most cases and less than 6 mm in 2 of these lesions. Four of 10 lesions were asymmetric, whereas borders were irregular in only 2 cases. Color was homogeneous in most lesions, being predominantly brown in 6 cases and blue and pink in 2 lesions each. In contrast, most melanomas in the other 2 groups fulfilled ABCD (asymmetry, borders irregular, color variegated, and diameter >6 mm) clinical criteria for suspect lesions. Clinical ulceration was present in 5 NM, 4 SSMNod, and 2 SSMBlue lesions.

DERMOSCOPIC FINDINGS

Two pure NMs were hypomelanotic, and the remaining 28 lesions were pigmented. The most relevant dermoscopic findings are given in Table 2. Although NMs usually were clinically symmetric, an asymmetric color and pattern distribution was observed in all lesions at dermoscopy. All lesions exhibited at least 3 colors, but the numbers of colors and structures were significantly lower in the NM group than in the SSM groups. Total dermoscopic scores (ABCD and 7-point checklist) were lower for NMs than for the other 2 groups, and differences were statistically significant when comparing pure NMs with SSMNod lesions.

Table 3. Relevant Confocal Findings

<table>
<thead>
<tr>
<th>Confocal Feature</th>
<th>NM (n=10)</th>
<th>SSMNod (n=10)</th>
<th>SSMBlue (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermal layers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epidermal pattern</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Honeycomb pattern</td>
<td>8</td>
<td>2a</td>
<td>1</td>
</tr>
<tr>
<td>Broadened</td>
<td>7</td>
<td>2a</td>
<td>0</td>
</tr>
<tr>
<td>Cobblestone pattern</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Disarranged</td>
<td>2</td>
<td>8a</td>
<td>8</td>
</tr>
<tr>
<td>Pagetoid cells</td>
<td>6</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Mild</td>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Moderate to intense</td>
<td>1</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Roundish</td>
<td>2</td>
<td>8a</td>
<td>10</td>
</tr>
<tr>
<td>Dendritic</td>
<td>6</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Basal layer and dermoepidermal junction</td>
<td>1</td>
<td>5</td>
<td>10b</td>
</tr>
<tr>
<td>Visible papilla</td>
<td>7</td>
<td>9</td>
<td>2b</td>
</tr>
<tr>
<td>Edged papilla</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Nonedged papilla</td>
<td>1</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Diffuse atypical cells</td>
<td>7</td>
<td>9</td>
<td>2b</td>
</tr>
<tr>
<td>Marked cell atypia in basal layer</td>
<td>8</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Superficial dermis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single nucleated cells</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Plump cells</td>
<td>7</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Clusters of cells</td>
<td>7</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Dense regular nests</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Dense irregular nests</td>
<td>6</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Cobblestone pattern</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Enlarged vessels</td>
<td>9</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Bright fibrillar structures</td>
<td>9</td>
<td>2a</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: NM, pure nodular melanoma; SSMNod, superficial spreading melanoma with a blue palpable area not yet nodular; SSMBlue, SSM with a nodular area.

aSignificant (P<.05) compared with NM.
bSignificant (P<.05) compared with SSMNod.

RCM AND HISTOPATHOLOGIC CORRELATES

Confocal aspects observed in the epidermal layers, dermoepidermal junction, and superficial dermis are given in Table 3.

EPIDERMAL LAYERS

Eight NMs exhibited a honeycomb pattern in the epidermal layers, with only 2 lesions demonstrating a disarranged pattern, observed in 8 SSMNod (P=.01) and SSMBlue lesions. Seven NMs (70%) had an atypical broadened honeycomb pattern that consisted of polygonal cells with black nuclei and a bright thick border (Figure 1B), observed also in 2 SSMNod lesions (P=.04). Pagetoid cells within the epidermis were more frequently observed in SSMs. In most NMs, pagetoid...
melanocytosis was constituted by few focally distributed small dendritic cells, whereas 6 SSM
Nod lesions exhibited numerous round and dendritic pagetoid cells distributed throughout the entire lesion (Figure 2B and Figure 3B), showing statistical differences between both groups when round pagetoid cells were considered ($P=.01$). Pagetoid cells seen at RCM correlated with histologic pagetoid spreading in 22 lesions (73%), with good correlation in the evaluation of round cells ($\kappa=0.70$; $P<.001$). Dots observed at dermoscopy correlated with pagetoid cells seen at RCM in 24 lesions (80%) and with histologic pagetoid spreading ($\kappa=0.55$; $P=.004$). At confocal microscopy, intraepidermal bright granular particles between epidermal cells were present in 5 SSM
Nod lesions and were not found in the NM group ($P=.02$). They correlated with more pigmented lesions and may correspond to transepidermal melanin loss (free melanin). At RCM, comparison of SSM
Nod and SSM
Blue lesions demonstrated no significant differences in upper layers. The predominant epidermal pattern was disarranged, and pagetoid cells were present in all lesions.

**DERMOEPIDERMAL JUNCTION**

Dermal papilla were rarely visible in NMs and in only half of the SSM
Nod lesions ($P=.14$). The nonvisibility of dermal papillae resulted at confocal microscopy in the sudden transition between epidermal layers and dermal structures, corresponding at histologic analysis with a thin flattened epidermis overlying the tumor burden ($\kappa=0.05$; $P=.004$). When present, dermal papillae were irregular in shape and distribution, showing nonedged contours corresponding to marked architectural disarrangement of the rete ridge. In contrast, dermal papillae were visible in all SSM
Blue lesions, similarly showing a nonedged aspect in all cases (Figure 4B) but in combination with an edged aspect in 4 lesions. In SSM lesions, the observation of dermal papillae at the dermoeipidermal junction at RCM correlated with the presence of a network at dermoscopy ($\kappa=0.04$; $P=.005$) and with the presence of elongated rete ridges ($\kappa=0.06$; $P=.004$) in histologic sections (Figure 4D).

Pleomorphic cells distributed in sheetlike structures were present at the dermoeipidermal junction and in the
superficial dermis in 7 NM and 9 SSMNod lesions (Figure 5C). Cells were large and markedly atypical in most cases (Table 3).

**DERMIS**

In both NM and SSMNod lesions, clusters of cells were visible in the dermis in 70% of cases. The NM group usually demonstrated both dense dishomogeneous clusters (6 lesions) (Figure 6A) and cerebriform clusters (6 lesions) (Figure 6C). In SSMNod and SSMBlue lesions, nests were mostly dense and dishomogeneous (Figure 2C and Figure 4C), with a single SSMNod also exhibiting cerebriform clusters (Figure 3C). At dermoscopy, globules showed good correlation with histopathologic dermal nests ($\kappa=0.05; P=.01$). In 22 lesions (73%), dense nests seen at RCM correlated with dermal aggregates of cells in papillary dermis. The observation of cerebriform clusters at RCM was associated with melanomas with a nodular pattern and deep tumoral infiltration ($\kappa=0.05; P=.001$). Single nucleated cells were observed in all lesions, corresponding to atypical melanocytes infiltrating the dermis (Figure 4C). Plump, bright, irregularly shaped cells with ill-defined borders and nonvisible nuclei corresponding to melanophages were present in 7 NM lesions and in 7 SSMNod lesions, and in all SSMBlue lesions. Refractive fibrillar structures gathered into large fasciae surrounding aggregates of cells were more frequently present in 9 NM compared with 2 SMMNod lesions and never seen in SSMBlue lesions ($\kappa=0.40; P=.005$). Moreover, enlarged vessels were present in 9 NMs (90%) (Figure 5B and Figure 6C), 7 SSMNod (Figure 3C), and 5 SSMBlue lesions (Table 3). Dermoscopic vessels correlated with the presence of vessels at RCM ($\kappa=0.40; P=.01$) that corresponded to dilated vessels under the epidermis ($\kappa=0.40; P=.01$). However, vessels visualized at dermoscopy and RCM were often more prominent than those observed in histopathologic sections.

**COMMENT**

In our study, dermoscopic evaluation revealed characteristic features in NM lesions and significant differ-
ences compared with SSM groups. The dermoscopic pattern was nonspecific according to classic dermoscopic pattern analysis; however, the lesions exhibited at least 3 dermoscopic structures. In contrast to clinical evaluation, dermoscopic analysis demonstrated enough criteria for malignancy in most NMs. The presence of a blue-white veil and vessels was more frequent in the nodular groups (NM and SSMNod) than in SSMBlue lesions, demonstrating that NMs exhibit dermoscopic findings associated with deep tumoral extension.

Like dermoscopy, RCM is a complementary imaging technique that enables the study of skin tumors in the horizontal plane. Reflectance-mode confocal microscopy enables a quasi-histologic resolution and also a different architectural view of the tumor that can be better evaluated in combination with the dermoscopic images. In addition, dermoscopy enables localization of confocal images in the tumor and renders the area for exact pathologic correlation in the vertical plane.

Reflectance-mode confocal microscopy is useful in the diagnosis of skin tumors including melanocytic lesions and basal cell carcinoma. Several studies have attempted to describe confocal features for the characterization of melanocytic and nonmelanocytic skin tumors and have performed dermoscopic and histopathologic correlations. A diagnostic semi-quantitative algorithm for RCM evaluation of clinically and dermoscopically equivocal melanocytic lesions was recently proposed. Two major confocal criteria (presence of nonedged papilla and cytologic atypia) and 4 minor confocal criteria (presence of roundish cells in the superficial layers, pagetoid cells widespread throughout the lesion, cerebriform clusters, and nucleated cells within the dermal papilla) were associated with malignancy. The sensitivity and specificity of confocal features for the diagnosis of melanoma were later evaluated in a further study by 2 blinded expert observers.

Previously, nodular melanoma was not investigated using RCM. In the present pilot study, pure NMs exhibited some differential features at RCM compared with SSMs. These differences often correlated with dermoscopic and histopathologic findings.

Within the epidermis, NMs lacked characteristic features of melanoma such as epidermal disarrangement and pagetoid spreading, usually showing a honeycomb pattern or a peculiar broadened pattern consisting of po-
lygonal cells with black nuclei and bright thick borders. In contrast, at confocal microscopy, SSM groups showed similar epidermal patterns characterized by a mostly disarranged pattern and the presence of moderate to intense pagetoid cells.

At the dermoepidermal junction, the nodular component of both NMs and SSMNod lesions exhibited similar features. Immediately below the epidermal layers, the typical papillary architecture was not visible in the nodules, corresponding to the epidermal flattening caused by the massive proliferation of malignant cells in the dermis. Markedly pleomorphic cells with bright cytoplasm and dark nuclei were present both in the basal layer, sometimes distributed in sheetlike structures, and in the upper dermis, isolated or aggregated in dishomogeneous clusters, in both NM and SSMNod lesions, whereas deep, amorphous, hyporeflective nests, called “cerebriform nests,” were more frequently observed in NMs, correlating with deep tumoral infiltration. In contrast, in SSMBlue lesions, the rete ridge was markedly disarranged but grossly preserved, resulting in the frequent observation of irregularly sized and shaped nonedged papillae in combination with marked cytologic atypia. Nucleated cells corresponding to malignant melanocyte infiltration were also observable in SSMBlue lesions, usually in combination with dishomogeneous aggregates of atypical cells.

As a new RCM feature of nodular melanomas, we found in the upper dermis some bright fibrillar structures bunched in large bundles and delimiting aggregates of atypical cells. Histopathologic correlation was difficult given the few lesions; however, they probably correspond to compacted collagen surrounding the tumoral mass. Plump cells were present in most lesions and correlated with dermal macrophages, usually associated with a moderate degree of inflammation. Moreover, NM and SSMNod lesions exhibited enlarged and tortuous vessels in most cases but were less frequent in SSMBlue lesions, indicating the presence of prominent neovascularization in thicker and more advanced lesions.

Nodular melanomas and SSMNod showed similar confocal and histopathologic features in the dermal component, characterized by prominent cellularity and mod-
erate inflammatory infiltrate with the absence of regression. In contrast, within the epidermis, patterns of substantial intraepidermal growth such as marked pagetoid spread and epidermal disarrangement were present in lesions with a superficial spreading component (SSM$_{nod}$ and SSM$_{blue}$ lesions), but these were lacking, except for few sporadic pagetoid cells, in NMs. Thus, from these findings in SSMs, the vertical growth seemed to arise within a predominantly horizontal growing population that is still found in the epidermis overlying the nodule, in contrast to a vertical growth of pleomorphic cells from the beginning in pure NMs.

To our knowledge, histopathologic studies that systematically compared NMs and SSMs have not been reported in the recent literature. However, it is classically accepted that NMs demonstrate vertical growth without evidence of an associated radial growth phase beyond the width of 3 rete ridges beyond the invasive component in any section. Intraepidermal spread in NMs is absent or limited to an area above the nodule, whereas SSMs characteristically exhibit prominent pagetoid spreading. In our pilot study, we were able to demonstrate these differences in vivo.

Confocal and histopathologic correlation enables in vivo visualization of some characteristic histologic features in the epidermis and superficial dermis, whereas aspects deeper than 300 µm were not visible owing to technical limitation. In this study, we confirmed some already described RCM criteria for SSM and their dermoscopic and histologic correlation. In addition, we characterized NMs using dermoscopy and RCM, demonstrating differential features between pure NMs and SSMs, with a subgroup of lesions in the middle of the spectrum (SSM$_{nod}$).

Possible limitations of the present study are the few pure NMs and that clinicians who performed the confocal and histopathologic evaluations were not blinded to the dermoscopic images. Because the correlation of RCM, dermoscopy, and histopathologic analysis was performed by a single, nonblinded observer (S. Segura), the analysis of agreement between these methods was performed as a preliminary exploration, and further studies are required to confirm the findings.

Reflectance-mode confocal microscopy seems to be a promising technique for the study of melanocytic lesions and nodular-type malignant melanoma. However,
larger studies with pure NMs should be done to better characterize these lesions, and genetic studies should be performed to enable further understanding of the histogenetic subtypes and biological behavior of malignant melanoma.

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Author Contributions: Dr Segura had full access to the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.  
Study concept and design: Segura and Pellacani.  
Acquisition of data: Segura, Pellacani, Longo, Bassoli, Guitera, and Palou.  
Analysis and interpretation of data: Segura, Pellacani, Puig, and Malvehy.  
Drafting of the manuscript: Segura.  
Critical revision of the manuscript: Pellacani, Puig, Guitera, Menezes, Seidenari, and Malvehy.  
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REFERENCES

Error in k Values. In the article titled “In Vivo Microscopic Features of Nodular Melanomas: Dermoscopy, Confocal Microscopy, and Histopathologic Correlates,” by Segura et al, published in the October issue of the Archives (2008;144[10]:1311-1320), several k values were reported incorrectly in the “Results” section on pages 1314 and 1315. On page 1314, left column, lines 10 through 13 should have read as follows: “Dots observed at dermoscopy correlated with pagetoid cells seen at RCM in 24 lesions (80%) and with histologic pagetoid spreading (k=0.50; P=.004).” On the same page, right column, “Dermoeipidermal Junction” subsection, lines 2 through 7 should have read as follows: “The nonvisibility of dermal papillae resulted at confocal microscopy in the sudden transition between epidermal layers and dermal structures, corresponding at histologic analysis with a thin flattened epidermis overlying the tumor burden (k=0.50; P=.004).” Further down, lines 13 through 19 should have read as follows: “In SSM lesions, the observation of dermal papillae at the dermoepidermal junction at RCM correlated with the presence of a network at dermoscopy (k=0.40; P=.005) and with the presence of elongated rete ridges (k=0.60; P=.004) in histologic sections (Figure 4D).” On page 1315, left column, “Dermis” subsection, lines 8 through 10 should have read as follows: “At dermoscopy, globules showed good correlation with histopathologic dermal nests (k=0.50; P=.01).” On the same page and in the same column, lines 12 through 15 should have read as follows: “The observation of cerebriform clusters at RCM was associated with melanomas with a nodular pattern and deep tumoral infiltration (k=0.30; P=.001).” On the same page, right column, lines 7 through 9 should have read as follows: “These structures corresponded to compact collagen bundles distributed around a tumoral mass (k=0.40; P=.005).”