False-Negative Cases on Confocal Microscopy Examination: A Retrospective Evaluation and Critical Reappraisal

Valeria Coco, Francesca Farnetani, Anna Maria Cesinaro, Silvana Ciardo, Giuseppe Argenziano, Ketty Peris, Giovanni Pellacani, Caterina Longo

Dermatology Unit, Catholic University, Rome, Dermatology Unit and Pathology Unit, University of Modena and Reggio Emilia, Modena, Dermatology Unit, Second University of Naples, Naples, and Dermatology and Skin Cancer Unit, Arcispedale Santa Maria Nuova-IRCCS, Reggio Emilia, Italy

Key Words
Confocal microscopy · Dermoscopy · Melanoma · Skin cancer · Nevi · Histopathology

Abstract
Background: Confocal microscopy is a second-level examination for dermoscopically equivocal melanocytic lesions. However, the number of false-negative cases on confocal microscopy and the scenarios in which confocal microscopy may fail have not been fully elucidated. Objective: To calculate the percentage of false-negative melanomas upon reflectance confocal microscopy examination in a large series of cases. Methods: A retrospective analysis on 201 melanomas, evaluated for dermoscopic/confocal criteria of melanoma, was carried out. Results: Twenty-three melanomas out of 201 cases (11.4%) revealed a low 7-point checklist score. On confocal examination, 22 out of 23 lesions have been diagnosed correctly as melanomas. Only 1 lesion did not display melanoma features, neither upon dermoscopy nor upon confocal microscopy examination. Seven lesions out of 201 cases (3.5%) were judged as negative on confocal examination, even if 6 of them were diagnosed as melanomas by clinical and/or dermoscopic evaluation. After histopathological revision, these cases were grouped into 5 categories: (1) amelanotic melanoma (n = 1), (2) hyperkeratotic melanomas (n = 2), (3) lentiginous melanomas (n = 2), (4) melanoma with small pagetoid cells (n = 1), (5) spitzoid melanoma (n = 1). Conclusion: Confocal and dermoscopic examination, along with patient-related information and clinical history, can lead to an optimal patient management.

Introduction

Data on melanoma from Cancer research UK calculated that in the time frame 1975–2010, the age-standardized incidence rate in the UK rose from 3.2/100,000 to 17.2/100,000 [1].

The key for preventing melanoma-related death is the detection of the tumor in its early stage where surgical excision is curative [2], since available therapies are still not optimal for advanced diseases.

Thus, noninvasive diagnostic tools have been developed and tested in clinical settings. The currently accept-

However, a percentage of melanomas can be difficult to be diagnosed even on dermoscopic grounds, and they can represent a gray zone. To narrow this gray zone, reflectance confocal microscopy (RCM) has been introduced in the clinical setting because it provides high-magnification images of a given skin lesion at a cellular resolution that is similar to those of histopathology. RCM has been developed as an adds-on tool for dermoscopically equivocal lesions.

Recent longitudinal prospective studies demonstrated that the systematic application of RCM as a second-level examination in skin oncology influences the lesion outcome in two thirds of cases, improving diagnostic accuracy [8, 9]. Although in these studies few false-negative results were found, the risk to miss confocal featureless melanomas should be considered.

In the present study we aimed to calculate, on a retrospective basis, the number of false-negative confocal cases and to investigate the reasons for their misdiagnosis. This analysis would be useful for clinicians to manage pigmented lesions according to a safer decision model.

**Materials and Methods**

For further details, see the online supplementary materials (see www.karger.com/doi/10.1159/000443637 for all online suppl. material) [10–14] (fig. 1).

**Results**

**Study Population**

A total of 201 biopsy-proven melanomas were evaluated (mean Breslow thickness of 0.9 mm; range from 0 to 12 mm). The study population included 83 women (41.3%) and 118 men (58.7%), with a mean age of 63 years (range from 26 to 94 years old). Concerning the body site, 103 melanomas (51.2%) were located on the trunk, 35 (17.4%) on the lower limbs, 25 (12.4%) on the upper limbs and 38 (19%) on the head/neck.

![Flowchart of Materials and Methods](fig. 1)
Dermoscopically Negative Cases

Twenty-three melanomas out of 201 cases (11.4%) revealed a low 7-point checklist score (from 0 to 2) and thus were judged to be ‘featureless’ or ‘equivocal’ lesions on dermoscopy but not falling into the category of clear-cut melanomas. On confocal examination, in 22 out of 23 lesions, melanoma clues have been detected. Only 1 lesion was not displaying melanoma features upon confocal microscopy examination. More specifically, the lesion was excised with the suspicion of an epithelial tumor because of its clinical aspect even if diagnostic features of basal cell carcinoma upon RCM evaluation were absent. Histology revealed a melanoma in situ arising on a previous compound nevus, with small pagetoid cells in the epidermal layer (fig. 2).

Lesions That Were Judged as Negative upon RCM Analysis

Seven lesions out of 201 (3.5%) were typified by a clinical and/or dermoscopic suspicion of melanoma or other epithelial tumor diagnosis and then sent for RCM examination (table 1). All these lesions turned out to be negative on confocal examination (absence of diagnostic RCM criteria for melanoma), but they were diagnosed as melanomas on histopathology (mean Breslow thickness of 0.36 mm). Almost all melanomas lacked evident RCM criteria; only in 1 case were there few atypical cells at the dermoepidermal junction (DEJ), where-

Table 1. False-negative cases on confocal microscopy examination

<table>
<thead>
<tr>
<th>Case</th>
<th>Clinical examination</th>
<th>Dermoscopy</th>
<th>RCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Case 2</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Case 3</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Case 4</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Case 5</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Case 6</td>
<td>+</td>
<td>+/-</td>
<td>–</td>
</tr>
<tr>
<td>Case 7</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

Fig. 2. a A large-sized lesion on the right arm of a 69-year-old man. b Dermoscopically it shows a reddish and whitish background and linear/arborizing vessels resembling an epithelial tumor. c On RCM evaluation no specific features for basal cell diagnosis can be found, but only few pagetoid cells are barely visible (arrows) along with severe reflective fibrosis (d). e Histological diagnosis reveals an in situ amelanotic melanoma. f At higher magnification small pagetoid cells and junctional nests with atypical melanocytes are observed.
as 2 cases revealed a nonspecific pattern at the DEJ, but their number and extent were not suggestive of melanoma diagnosis [15].

**Histopathological Nonblinded Case Review**

To better understand why these melanomas appeared as false negative on RCM, we reviewed the histological sections with a dermatopathologist (table 2). False-negative confocal cases can be classified into 5 categories: (1) amelanotic melanoma (n = 1); (2) hyperkeratotic melanomas (n = 2); (3) lentiginous melanomas (n = 2); (4) melanoma with small pagetoid cells (n = 1); (5) spitzoid melanoma (n = 1).

**Amelanotic Melanoma.** The patient was a 69-year-old man, and the lesion was located on his right arm. Clinically and dermoscopically it appeared as an epithelial tumor because of its reddish and whitish background with linear and arborizing vessels. On RCM evaluation, no basal cell carcinoma diagnostic features were found. On the other side, we could not diagnose it as a melanoma because few pagetoid cells were barely visible along with severe fibrosis. The absence of any RCM diagnostic criteria for melanoma or basal cell carcinoma prompted the excision of the tumor. In fact, the ‘absence’ of any RCM criteria should always be interpreted with caution because it could be related to bad imaging, lack of adequate training or presence of focal diagnostic clues that could be missed. Histological diagnosis confirmed that it was an in situ amelanotic melanoma (fig. 2).

**Hyperkeratotic Melanomas.** The two patients were 46 and 65 years old, and the lesions were located on the left leg and left arm, respectively. These cases were negative on RCM because they had a thick hyperkeratosis that hampered the RCM imaging of the DEJ and the upper dermis. Histological evaluation confirmed that one of the lesions had a spitzoid pattern with few pagetoid cells in the epidermis, while the second revealed no pagetoid infiltration and deeply located tumoral nests. The lesions

<table>
<thead>
<tr>
<th>RCM criteria</th>
<th>Histological diagnosis</th>
<th>Breslow thickness, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amelanotic melanoma Case 1</td>
<td>Presence of few hyporefractive pagetoid cells focally located; severe fibrosis</td>
<td>0</td>
</tr>
<tr>
<td>Hyperkeratotic melanoma Case 2</td>
<td>Typical pattern of a seborrheic keratosis; the DEJ and the upper dermis were not visible</td>
<td>0.3</td>
</tr>
<tr>
<td>Hyperkeratotic melanoma Case 3</td>
<td>No pagetoid or atypical cells; the DEJ and upper dermis were not observable because of a thick epidermis</td>
<td>1.3</td>
</tr>
<tr>
<td>Lentiginous melanoma Case 4</td>
<td>Typical pattern of a compound nevus</td>
<td>0.5</td>
</tr>
<tr>
<td>Lentiginous melanoma Case 5</td>
<td>Pattern of a compound nevus without cytological atypia</td>
<td>0</td>
</tr>
<tr>
<td>Melanoma with small pagetoid cells Case 6</td>
<td>Absence of pagetoid cells in the epidermis; presence of focal disarrangement at the DEJ with inflammation</td>
<td>0.44</td>
</tr>
<tr>
<td>Spitzoid melanoma Case 7</td>
<td>Absence of cytological atypia; a regular meshwork pattern was present with elongated interpapillary spaces</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 2. RCM criteria and histological characteristics of false-negative melanomas on confocal examination**
were excised because of their clinical aspects and dermoscopic examination, and they had a Breslow thickness of 0.3 and 1.5 mm, respectively (fig. 3).

Melanomas with Lentiginous Pattern. The first patient was a 55-year-old man, and the lesion was located on his back. On clinical examination the lesion was larger and darker compared to his other nevi. Confocal examination showed the presence of a clod pattern as seen usually in compound nevi. The pathologist diagnosed it as lentiginous melanoma with a Breslow thickness of 0.5 mm and with few pagetoid cells in the epidermis; the lesion did not fulfill all histopathological criteria for melanoma diagnosis since it could also be interpreted as dysplastic nevus. However, the large lesion size favored the diagnosis of melanoma (fig. 4). The second patient was a 60-year-old woman who presented a large lesion on the back. Dermoscopically the lesion had a slightly atypical network and peripheral area of regression, with blue-gray globules. On RCM evaluation a pattern of a compound nevus was present, without severe cytological atypia. Reviewing the histology it was described as a lentiginous melanoma in situ with single cell pattern of growth that in some parts resembled a lentigo maligna with a thinned epidermis. Few pagetoid cells were also present that further supported the diagnosis of melanoma.

Melanoma with Small Pagetoid Cells. In this case, melanoma showed on histopathology a pagetoid infiltration composed of single and small cells. We did not catch these cells on confocal images, probably because they were too small and focally present on the lesion. At the DEJ it was possible to detect inflammation and change of the architecture, but it was not sufficiently alarming to address the diagnosis of melanoma. The lesion was located on the right leg of a 70-year-old woman. At the first visit it was sent for follow-up monitoring, but after 6 months the lesion was excised because it was enlarging in size (fig. 5).

Spitzoid Melanoma. The lesion was located on the left breast of a 43-year-old woman. Clinically it was an ugly duckling lesion with irregular pigmentation. On dermoscopy examination it was clearly asymmetric with a dark and a lighter pigmented area. On RCM evaluation neither alterations in cytology nor in the architecture were found. A meshwork pattern was present with elongated interpapillary spaces as it is frequent in this special body site. Histology confirmed that no pagetoid infiltration was present and that the lesion had a spitzoid pattern that could also be classified as a dysplastic nevus. The major
**Fig. 4.**

a On clinical examination, the lesion is larger and darker compared to the surrounding other nevi. b Dermoscopically, it reveals a structureless pattern. c Confocal examination shows a regular epidermal pattern and the presence of a clod pattern (arrows; d) as seen usually in compound nevi. e Histopathology depicts a lentiginous melanoma with a Breslow thickness of 0.5 mm. f At higher magnification, very few pagetoid cells can be seen.

**Fig. 5.**

a Clinically, the lesion is a large pigmented flat tumor on the right leg of a 70-year-old woman that was enlarging in size over a 6-month follow-up. b Dermoscopically, it has a slightly atypical network and different grade of pigmentation from the center to the periphery. c Upon RCM evaluation at the DEJ, it was possible to detect inflammation and loss of the architecture, but it was not alarming enough to address the diagnosis of melanoma. d On histopathology, pagetoid spread composed by single and small cells was present.
Confocal Microscopy and False-Negative Cases

criteria for melanoma diagnosis were the presence of the irregular distribution of pigmentation and lesion size (fig. 6).

Discussion

In the current study, we quantified and characterized the RCM false-negative melanomas, in a routine clinical setting, in 3.5% of a large series of melanomas. False-negative cases on RCM examination fall into 5 main categories of melanoma subtypes.

The first category includes 1 case of amelanotic melanoma: this type of melanoma represents a diagnostic challenge both clinically and dermoscopically. On confocal microscopy there are few studies that reported characteristics of this type of melanoma [16–20]. Recently, hyporeflective pagetoid cells have been claimed as a new clue in the diagnosis of amelanotic melanoma [21]. In the same paper, the authors suggest that a solitary hypopigmented lesion, in the absence of any specific nonmelanocytic or melanocytic features, should always be excised due to the probability of melanoma diagnosis.

The second category of false-negative cases regards melanomas with a hyperkeratotic surface: the laser power employed by RCM (near-infrared wavelength of 830 nm) reaches the maximum depth of 200–300 μm, which in normal skin corresponds to the papillary dermis. It is intuitively obvious that the more hyperkeratosis, the more RCM imaging is limited [22], because it may hamper the correct visualization of the DEJ and upper dermis.

Another challenging group of tumors on RCM examination is represented by the lentiginous melanoma subtype: this entity includes borderline lesions with a lentiginous proliferation of single melanocytes along the DEJ. Of note, this entity can also be challenging in histology because lentiginous melanomas may show significant overlapping features with atypical lentiginous nevus [23–25]. Awareness of the size of the lesion is diagnostically important, as in our cases, in which the overall clinical pictures along with the presence of subtle histopathological criteria were crucial to achieve the correct diagnosis.

In the present study, 1 melanoma with small pagetoid cells was also missed by RCM analysis. This variant of melanoma can be misdiagnosed by RCM because the reader may not readily detect single and small cells that...
can be easily misinterpreted as keratinocytes. Notably, also Guitera et al. [14] reported the occurrence of false-negative cases such as invasive melanomas with small cells.

The fifth RCM pitfall we met was a spitzoid melanoma: this is a difficult entity to diagnose and even histopathologically a clear-cut differentiation between benign and malignant spitzoid neoplasms is often challenging. Because of these difficulties in clinical and histopathological evaluation, surgical excision is always recommended for clinically atypical spitzoid lesions of childhood and for all spitzoid-looking lesions of adulthood [10, 26–28].

In conclusion, the results of our study demonstrate that RCM is a robust and effective tool for melanoma diagnosis with only few cases that are misclassified as benign. The limitation of our study is the retrospective analysis. Thus, there is no information on possible misdiagnosed and not excised melanomas lacking both clinical-dermoscopic features and confocal clues.

Since the proportion of false-negative melanomas upon dermoscopy was higher than upon confocal microscopy, and only 1 case was doubly negative, our findings further highlight that melanoma diagnosis is a complex process in which several factors such as patient-related information, clinical history, clinical aspects, dermoscopic and confocal features should be taken into account in order to reduce the risk of patient mismanagement.

**Acknowledgments**

This work was partially funded by Research Project NET-2011-02347213, Italian Ministry of Health.

**Statement of Ethics**

Institutional Review Board approval was not required.

**Disclosure Statement**

The authors report no conflicts of interest.

---

**References**


Confocal Microscopy and False-Negative Cases

Dermatology 2016;232:189–197
DOI: 10.1159/000443637


