

Role of *In Vivo* Reflectance Confocal Microscopy in the Analysis of Melanocytic Lesions

Worldwide melanoma incidence and mortality are increasing (1). Despite the ongoing research, advanced melanoma is still incurable; therefore, the most appropriate solution seems to be early detection combined with complete surgical excision (2). Since the diagnostic protocol of suspicious lesions includes a complete excision with safety margins (2), the problem of unnecessary scarring is significant. The real challenge in this case is to have a properly formulated diagnosis before acquiring a biopsy. Currently available non-invasive techniques are coherence tomography, digital dermoscopy, and reflectance confocal microscopy. All these techniques allow for a presumptive diagnosis, but the most promising results are provided by reflectance confocal microscopy.

Reflectance confocal microscopy (RCM) is an optical imaging technique that uses a laser diode as a source of coherent monochromatic light which penetrates the tissue and illuminates a single point. Light from the stimulated section is reflected and passes through a filter, thereby forming the image on the detector. This filter enables selective excitation of a particular point on which focus is achieved and rejects reflection from the out-of-focus area, thus obtaining a "confocal" image. Contrast is the result of differences in the refractive index of the cell organelles and microstructures, resulting in white structures on a black background. This technique allows, as opposed to conventional light microscopy, the analysis of sections obtained at a bi- or tri-dimensional level and controlling the depth of the field, permitting out-of-focus artifacts to be eliminated.

In dermatology, this technique is useful for both clinical and research purposes. It is the only technique that allows horizontal viewing of the skin up to the superficial dermis (approximately 300 μm , at a cellular level resolution (0.5-1.0 μm in the lateral dimension and 4.0-5.0 μm in the axial dimension) (3).

It allows both *in vivo* and *ex vivo* diagnosis, while providing the possibility for long-term monitoring.

It has proved to be especially valuable for *in vivo* examinations of melanocytic lesions, whereas melanin and melanosomes are a powerful source of contrast, allowing the individualization of melanocytic cells (4).

We report the case of a 65-year-old Caucasian woman who presented to the Dermatology Department of University of Modena and Reggio Emilia, Italy, for the examination of an atypical lesion, of unknown history, localized in the right preauricular area.

The patient's personal and family histories were negative for skin malignancies and for other significant medical history. The clinical presentation was highly indicative of malignancy, as it met all the ABCD clinical criteria: an asymmetric papule composed of two areas, one pigmented and another one hypopigmented, with ill-defined borders and a diameter of approximately 2 cm.

The dermatoscopic examination revealed an asymmetric multicomponent pattern with atypical network, structureless areas, peripheral irregular globules, and a blue-white veil.

Because clinical and dermatoscopic features pointed towards a suspicious lesion which was situated on the face, where unnecessary scarring is unwanted, reflectance confocal microscopy (RCM) examination was proposed and performed (VivaScope 3000; MAVIG GmbH, Munich, Germany) (5). It revealed the following features: the epidermis presented a disarranged pattern; the dermo-epidermal junction and superficial dermis presented a meshwork pattern with edged & non-edged papillae, non-homogenous junctional clusters, dense nests, dense & sparse nests, and atypical cells in a sparse distribution (Figure 1).

Figure 1. (A) Clinical examination of an atypical melanocytic lesion situated at the right preauricular area. (B) Dermatoscopic examination. (C) Confocal examination of dermo-epidermal junction and superficial dermis which reveals a meshwork pattern (yellow circle) with edged & non-edged papillae, non-homogenous junctional clusters (yellow star), dense

nests, dense & sparse nests (red star) and atypical cells in a sparse distribution (arrow).

The clinical and confocal data indicated a malignant melanocytic tumor, so an excisional biopsy with safety margins was performed. The histopathological report indicated superficial spreading melanoma with a Breslow of 0.55 mm and 0 mitosis/mm².

This case illustrates the important role confocal microscopy examination has in the management of melanocytic lesions situated in special areas like the face.

Reflectance confocal microscopy is an imaging technique that allows viewing the layers of the skin up to the superficial dermis and therefore turns out to be extremely useful in obtaining a pertinent diagnosis before acquiring a biopsy.

According to the data available so far, it was established that reflectance confocal microscopy increases the diagnostic accuracy for melanocytic lesions in both pigmented and hypopigmented lesions. In a study conducted by Borsari *et al.*, reflectance confocal

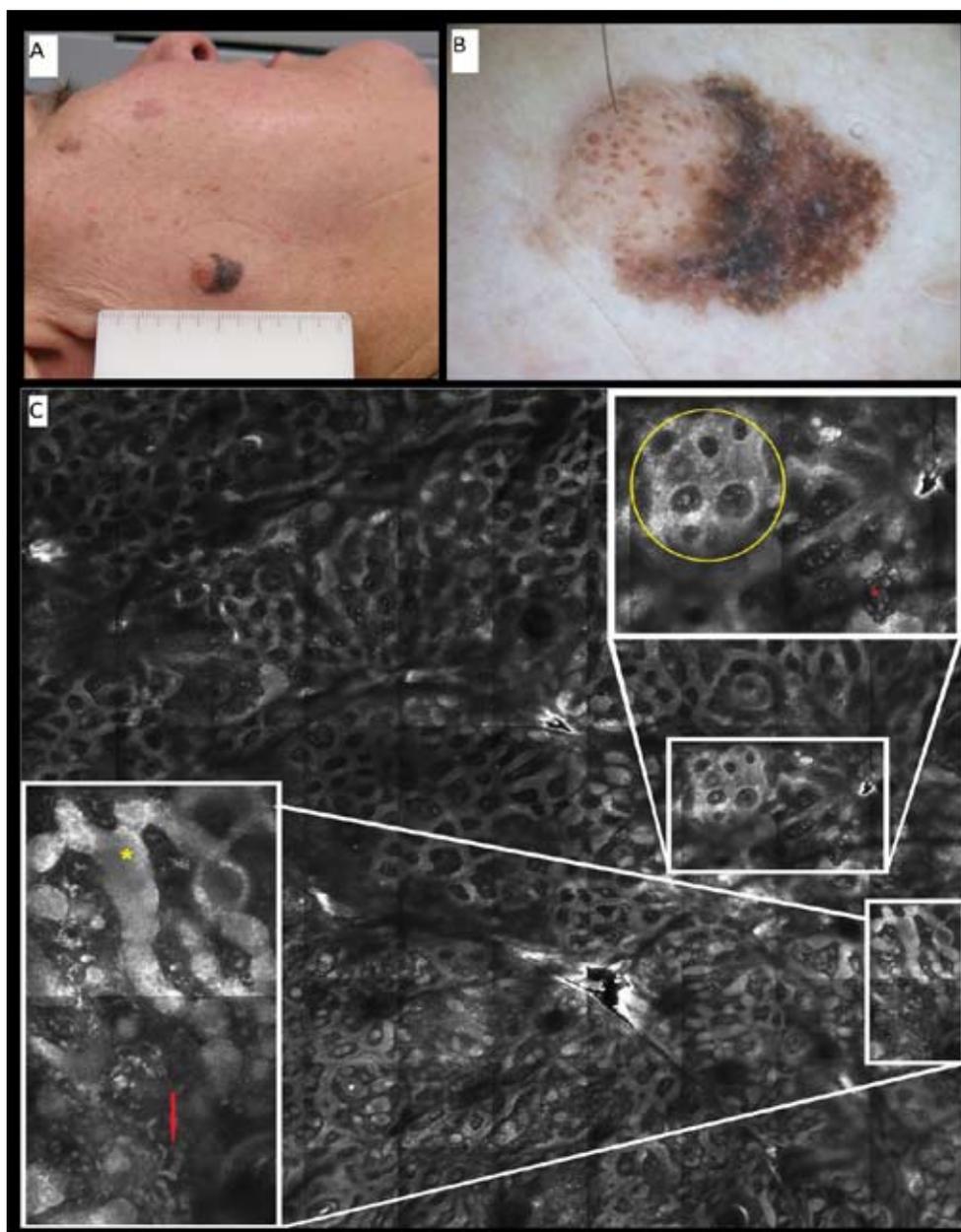


Figure 1.A. Clinical examination of an atypical melanocytic lesion situated at right preauricular area B. Dermatoscopic examination. C. Confocal examination of dermo-epidermal junction and superficial dermis which reveals a meshwork pattern (yellow circle) with edged & non-edged papillae, non-homogenous junctional clusters (yellow star), dense nests, dense & sparse nests (red star) and atypical cells in a sparse distribution (arrow).

microscopy proved to have a sensibility and specificity of 95.3% and 83.9%, respectively (6).

By improving the accuracy of clinical and dermatoscopic diagnosis, the reflectance confocal microscopy technique contributes to increasing the confidence of the clinical and dermatoscopic diagnosis (7). In this regard, confocal reflectance microscopy reduces unnecessary excisions, particularly in cases of damage to cosmetically important areas, such as the face or the neck, simultaneously detecting the malignant lesions that require a surgical approach, as seen in the case presented, where confirmation of the diagnosis by confocal microscopy allowed for a safe excision. In fact, the head and neck are the most appropriate body location for reflectance confocal examination, especially because RCM showed a high diagnostic accuracy for lesions located on sun-damaged skin, as these two areas frequently are (adjusted odds ratio (aOR), 2.13; 95% confidence interval (CI), 1.37-3.30; $P=.001$) (6).

Reflectance confocal microscopy is very helpful in the management of special lesions, like facial lentigo maligna melanoma. This type of lesion is considered to be a real challenge for the dermatologist because of its clinical and morphological features that are similar to other lesions such as solar lentigines and pigmented actinic keratoses. In this case, reflectance confocal excels at specificity of the diagnosis, but also at the ability to define the margins more accurately, permitting a pre-surgical mapping and for possibility of identifying the optimal site for biopsy (8,9).

By improving diagnostic ability, reflectance confocal microscopy technique may contribute to the selection of lesions that may be eligible for non-surgical treatment. Facial pigmented non-melanocytic macules like solar lentigo, flat seborrheic keratosis, lichen planus-like keratosis, and pigmented actinic keratosis can mimic a lentigo maligna, or even a lentigo maligna melanoma, but with the help of the RCM, an accurate diagnosis can be established, sparing the patient can be from unwanted facial scars using a non-surgical approach (laser, cryotherapy, imiquimod) (10,11).

Furthermore, reflectance confocal microscopy can be a valuable method for the monitoring of a skin lesion over time, especially melanocytic nevi, reducing unnecessary surgical excision, such as for patients with multiple atypical nevi that undergo multiple biopsies (12,13).

Like all other diagnostic methods, RCM has its limitations: palmoplantar lesions (due to thickened epidermis), ulcers or crusts on a large lesion, lesions localized in inaccessible regions such as interdigital space, nasal wing (3).

To summarize, reflectance confocal microscopy can improve clinical and dermatoscopic diagnosis of melanocytic lesions, detecting the lesions that need an invasive approach and preventing unnecessary excision. It has proven to be very helpful in the management of lentigo maligna and lentigo maligna melanoma, achieving high specificity in the diagnosis and simultaneously allowing an optimal approach. This technique can be a reliable bridge between dermatoscopy and histopathology, being able to provide an alternative to histopathological examination.

Special mention must be made of the factors that may change the result to a false negative such as hyperkeratosis, ulceration, or bleeding, so any results should be integrated with the rest of the patient's data.

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Received: April 25, 2017

Accepted: January 20, 2018

