In vivo examination of lentigo maligna and malignant melanoma in situ, lentigo maligna type by near-infrared reflectance confocal microscopy: Comparison of in vivo confocal images with histologic sections

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In vivo confocal microscopy can noninvasively image thin en face sections within living intact human tissue with high resolution and contrast. This evolving technique may provide clinicians with tools to help detect lentigo maligna lesion progression in vivo and may be important in defining tumor margins, thus providing a more definitive surgical eradication of lentigo maligna and malignant melanoma in situ, lentigo maligna type. We present a case of malignant melanoma in situ, lentigo maligna type, and we describe the images seen with confocal microscopy in correlation with routine histopathology. (J Am Acad Dermatol 2002; 46:260-3.)

Confocal reflectance microscopes can noninvasively image thin en face sections within living intact human tissue with high resolution and contrast; this is known as optical sectioning. The experimentally measured resolution of 0.5 to 1.0 μm (lateral) and 3 to 5 μm (axial) within the tissue allows for evaluation of nuclear, cellular, and tissue architecture of epidermis and the underlying collagen, connective tissue, circulating blood cells, and capillaries, without a biopsy.[1,2] The axial resolution defines the (noninvasive) optical section thickness; thus the confocal section thickness in vivo compares very well with the thickness of sections that are prepared for histopathology. The maximum depth of imaging is 350 μm in skin.[2]

The image contrast is mainly because of the detected variations in singly back-scattered light resulting from variations in the refractive index (n) of tissue microstructures.[1,3] The pigment melanin has a high refractive index (n = 1.70)[1] and thus acts as an endogenous stain, especially when imaging pigmented lesions such as lentigo maligna.[4]

We have previously hypothesized that lesions currently classified as lentigo maligna include 2 categories of lesions.[5] The first is a putative precursor lesion characterized histologically by atypical melanocytic hyperplasia, which we termed *lentigo maligna*. In addition to atypical melanocytic hyperplasia, the second category of lesions is characterized by pagetoid spread, confluence, and nesting of atypical melanocytes, which we designated as malignant melanoma in situ, lentigo maligna type. In our recently published study, we reported that the intraepidermal component of 42 consecutive cases of invasive malignant melanoma, lentigo maligna type showed features diagnostic of malignant melanoma in situ, lentigo maligna type, in the epidermis overlying the invasive dermal component.[6] We concluded that invasive lentigo maligna melanoma arises in association with those lesions that we have termed malignant melanoma in situ, lentigo maligna type, and less often from the lesions that only demonstrate lentigo maligna, which is characterized by atypical melanocytic hyperplasia. This finding suggests that melanoma in situ may be a necessary step in the progression of lentigo maligna and not only supports the distinction of these entities but also may have therapeutic implications.

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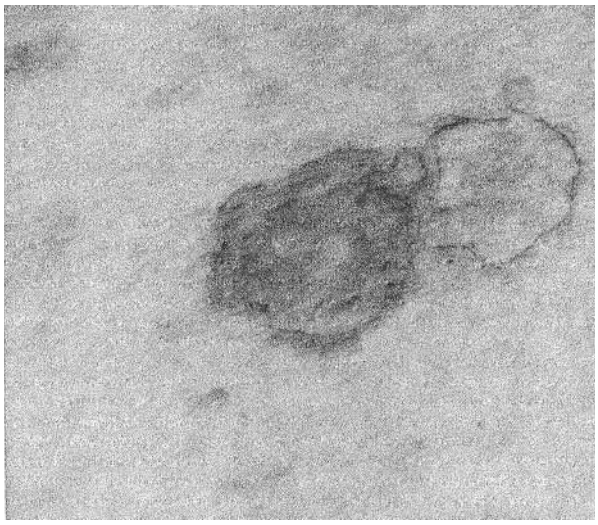

Fig 1. Note variably pigmented patch on chest. Clinical margin is delineated by Wood's lamp.

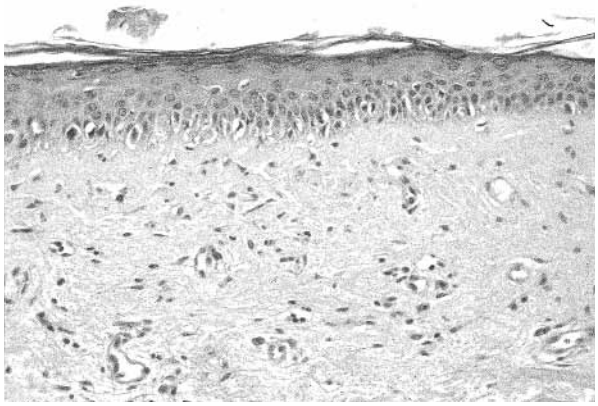

Fig 2. Lentigo maligna. Note atypical lentiginous melanocytic hyperplasia along dermoepidermal junction. (Hematoxylin-eosin–stained sections.)

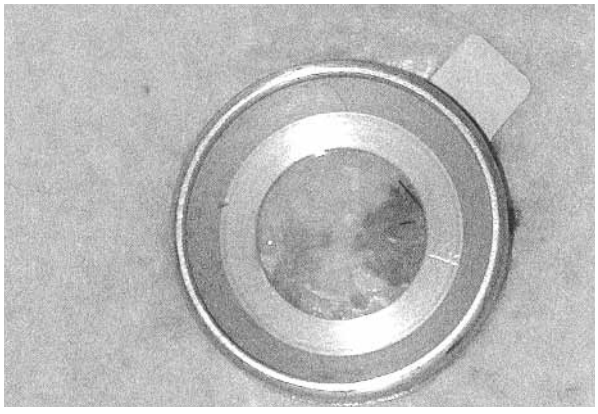

Fig 3. Confocal imaging. Tissue ring is applied on skin with clinical edge of lesion coinciding with center of ring, which is illuminated by light source.

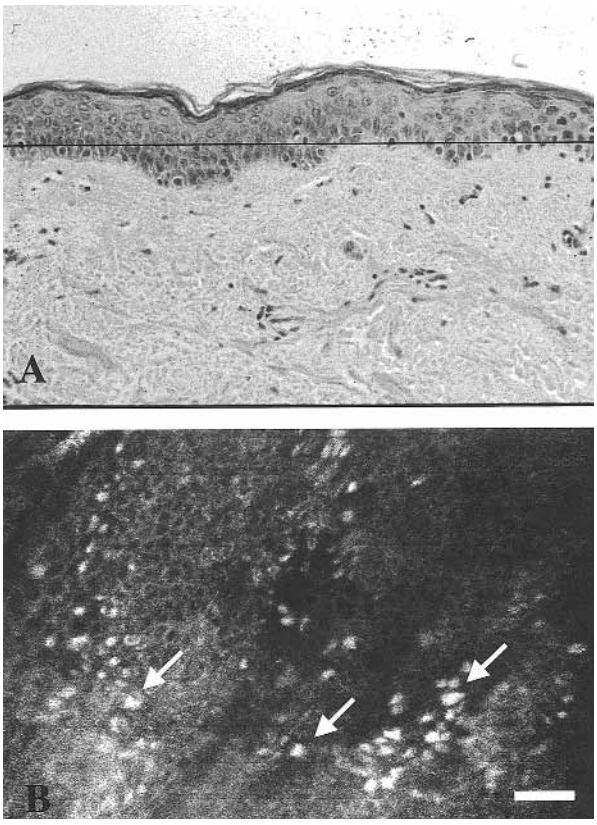

Fig 4. Clinical edge of lesion. Both hematoxylin-eosin–stained permanent sections (**A,** vertical plane) and confocal images (**B,** transverse plane) show increased number of atypical melanocytes at dermoepidermal junction, consistent with lentigo maligna. In confocal imaging, melanocytes (**B,** *arrows*) appear intensely bright compared with adjacent keratinocytes. Scale bar = 50 μm.

CASE REPORT

The patient was a 65-year-old white woman who presented with a pigmented lesion on the chest that had been present for several years (Fig 1). A biopsy specimen taken from the edge of the lesion revealed atypical melanocytic hyperplasia, consistent with lentigo maligna (Fig 2). In vivo confocal imaging was performed before surgical excision (Fig 3).

Confocal imaging was done starting from the clinically normal skin, 2 mm away from the clinically visible margin, and moving toward the center of the lesion. Confocal imaging was performed by using a commercially available confocal microscopy system (VivaScope, Lucid Inc, Hervietta, NY) that uses a low-power 830-nm diode laser and a water immersion objective lens (×30, 0.9 numerical aperture). At the clinically normal skin, melanocytes appeared to have a bright cytoplasm and to be small, with small nuclear to cytoplasmic ratio and relatively round nuclei. They were widely dispersed as single cells at

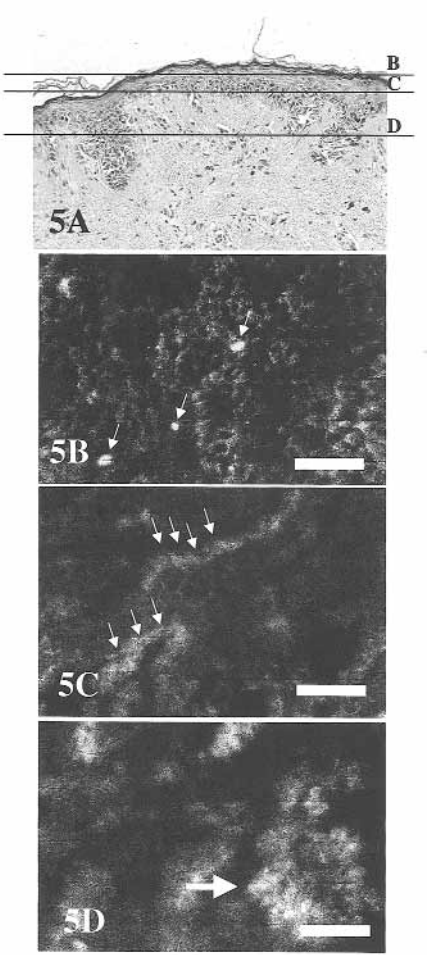

Fig 5. Correlation between hematoxylin-eosin–stained sections (**A,** vertical) and in vivo confocal images (**B, C, D,** transverse) of malignant melanoma in situ, lentigo maligna type. Note pagetoid spread of melanocytes in upper spinous layer (**A, B,** *arrows*), confluence of melanocytes replacing epidermal basal cell layer (**A, C,** *arrows*), and nesting of melanocytes (**A, D,** *arrows,* scale bar 50 μm).

the level of the basal cell layer. At the clinically visible margin, confocal imaging confirmed the presence of lentigo maligna, revealing an increased number of relatively larger melanocytes still arranged as single cells at the dermoepidermal junction (Fig 4). However, the center of the lesion demonstrated changes consistent with melanoma in situ with melanocytes at different levels of epidermis (pagetoid spread), becoming confluent in some foci with replacement of the basal keratinocytes, and forming discrete nests of atypical melanocytes in other foci (Figs 5 and 6). These melanocytes appeared brighter in color than the intervening basal keratinocytes, with large angulated nuclei, high nuclear to cytoplasmic ratio, and some dendritic processes (Fig 7).

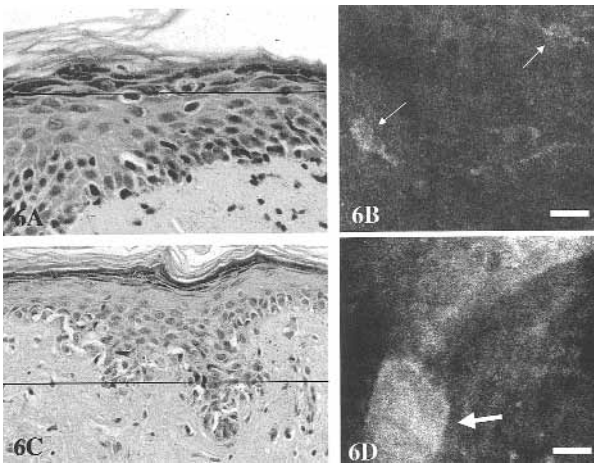

Fig 6. Correlation between hematoxylin-eosin–stained sections (**A, C,** vertical) and in vivo confocal images (**B, D,** transverse) of malignant melanoma in situ, lentigo maligna type. Higher magnification shows individual atypical melanocytes in upper malpighian and granular cell layers (**A, B,** *arrows*) and nesting of atypical melanocytes (**C, D,** *arrows,* scale bar 50 μm).

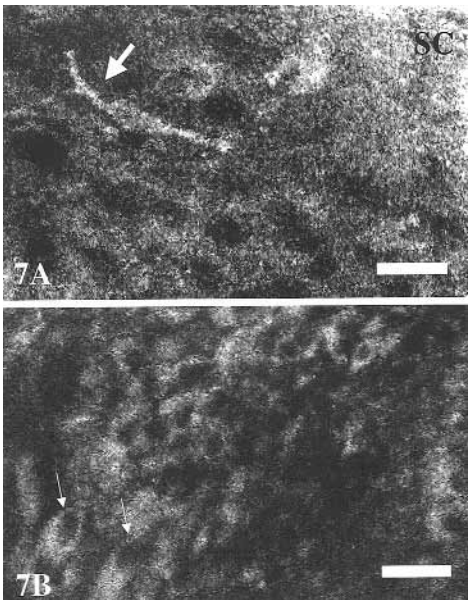

Fig 7. Confocal imaging. Some atypical melanocytes demonstrate prominent dendritic processes (**A,** *arrows,* scale bar 5 μm). On higher magnification, melanocytes appear to have large angulated nuclei with high nuclear to cytoplasmic ratio and intensely bright cytoplasm (**B,** *arrows*).

Examination of the routinely processed histologic sections after complete surgical excision confirmed the presence of malignant melanoma in situ, lentigo maligna type (Figs 5 and 6). The precursor lesion,

lentigo maligna, was present in the epidermis adjacent to the malignant melanoma in situ, lentigo maligna type.

DISCUSSION

The evolving technique of in vivo confocal microscopy is a high-resolution imaging tool that allows noninvasive optical sectioning of live human skin and other accessible tissues in real time.[1,2,7] It works by detecting single back-scattered photons from the illuminated tissue.[8] Therefore living skin may be imaged with no discomfort and without the need for anesthesia or administration of exogenous dyes. The image contrast is mainly the result of the detected variations in singly back-scattered light because of variations in the refractive index (n) of tissue organelles and other microstructures. In the skin, the pigment melanin has a high refractive index and thus acts as an endogenous contrast agent.[1,3]

To date, confocal microscopy has been used to define features of commonly encountered skin conditions.[4,9-12] Near infrared, reflectance confocal microscopy may provide clinicians with tools to help detect lesion progression in vivo. Furthermore, in vivo confocal microscopy may be important in defining tumor margins, thus providing a more definite surgical irradiation of lentigo maligna, malignant melanoma in situ, lentigo maligna type, and invasive lentigo maligna melanoma, as well as tissue-sparing in cosmetically sensitive areas.

The apparent lower risk of progression to invasive melanoma from lentigo maligna than from lentigo maligna melanoma in situ may allow patients and their physicians to make informed decisions about less aggressive therapy for lentigo maligna. However, one must always be cognizant of the problem of sampling error for incisional biopsies. The use of in vivo confocal microscopy in the examination of lentigo maligna may potentially eliminate this inherent problem of sampling error because the entire lesion can be evaluated before biopsy. This information will be important in guiding therapy.

One of the limitations of in vivo confocal microscopy is that imaging is limited to the level of the upper reticular dermis with a maximum depth of penetration of 350 μm.[2]

However, most diagnostic features of lentigo maligna and melanoma in situ, lentigo maligna type are intraepidermal, making these lesions relatively easily detectable by confocal microscopy.

In conclusion, the histologic details seen in confocal images of lentigo maligna and malignant melanoma in situ, lentigo maligna type, compared well with those seen in the corresponding routinely processed histologic sections, as demonstrated in our case. Confocal microscopy provides an unprecedented view of the histology of living tissue, in its native, dynamic state, in an entirely noninvasive manner. This approach offers a promising imaging technique for pigmented skin lesions, especially lentigo maligna and lentigo maligna melanoma, which are notorious for their subclinical extension.

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