Use of In Vivo Confocal Microscopy in Malignant Melanoma

An Aid in Diagnosis and Assessment of Surgical and Nonsurgical Therapeutic Approaches

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Background: Melanomas with poorly defined borders, lack of pigmentation, lentiginous extension, and location in cosmetically sensitive regions represent diagnostic and therapeutic challenges. Repeated surgical reexcisions are frequently required to achieve tumor-free margins. The use of reflectance mode confocal microscopy as an noninvasive method has shown to be a promising tool for diagnosing pigmented lesions in vivo.

Observations: We report 3 clinical cases of melanoma: amelanotic melanoma (case 1), locally recurrent melanoma (case 2), and lentigo maligna melanoma (case 3). In case 1, in vivo confocal microscopy was instrumental in making the diagnosis and in monitoring the response to imiquimod therapy for in situ residual disease. It was also used to successfully delineate preoperative surgical margins in cases 2 and 3.

Conclusion: As new methods for treating melanoma emerge and become more available, confocal microscopy can play a significant role by improving sensitivity in diagnosis, by increasing rates of successful initial excision, and by serving as a noninvasive means of monitoring therapy.

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The incidence and mortality of malignant melanoma continue to increase at a faster rate than those of any other cancer in the United States. Early detection of cutaneous melanomas remains one of the areas with a greater need for the development of techniques that can improve the current clinical diagnostic accuracy. As new noninvasive imaging techniques continue to emerge for the assessment of melanocytic neoplasms, the applicability of these instruments can be simultaneously expanded into several clinical scenarios. In particular, potential areas of application include early identification of amelanotic melanoma and delineation of appropriate surgical management in cases of poorly defined lesions. Such instruments can also be used as a means of monitoring response to noninvasive therapy.

Novel imaging modalities include epiluminescence microscopy,1 confocal scanning microscopy,2 high-resolution ultrasound,3 and spectrophotometric intracutaneous analysis.4 The use of reflectance mode confocal microscopy as a noninvasive method for evaluation of melanocytic lesions offers the highest level of resolution. To date, this technique has proved to be particularly useful in the setting of intraepidermal malignant melanomas and lentigo maligna.5,6 The 3 cases reported herein illustrate the implementation of in vivo confocal microscopy (IVCM) in challenging clinical scenarios.

REPORT OF CASES

CASE 1

A 58-year-old man presented with a 1-mm, nonulcerating, flesh-colored papule on the distal aspect of his right nasal wall that was in close proximity to the site of a previous skin flap. No pigmentation was noted (Figure 1A). The patient’s dermatologic history was significant for an invasive lentigo maligna melanoma (Clark level III, 0.3 mm deep) on his right nasal bridge 12 years previously that was surgically excised and repaired with a flap taken from his right cheek. Eight weeks before his current presentation, an invasive nonulcerated primary amelanotic malignant melanoma (Clark level IV, 1.25 mm thick) was surgically excised with 1-cm margins from his right cheek. A sentinel lymph node biopsy specimen was without evidence of metastasis. The patient’s family history included a sister with cutaneous mela-
nomia. A review of systems and a physical examination revealed no extracutaneous disease.

Visual examination revealed no areas of altered pigmentation. Because the patient’s recently diagnosed malignant melanoma was amelanotic, IVCM imaging of the lesion on the right nasal tip was performed. Suspicious architecture and melanocytic proliferation suggested a diagnosis of melanoma (Figure 1B and D). Findings of a subsequent skin biopsy confirmed the diagnosis of lentigo maligna melanoma in situ with adnexal involvement and a dermal microsatellite (Figure 1C and E). Mohs surgery was performed owing to the lack of clinical demarcation and appendageal involvement, but after 4 stages, the margins remained positive (Figure 2A). No invasive component was apparent, and the risk of deformity with further surgery was considerable. The patient preferred a less invasive treatment; therefore, a means of monitoring for relapse was needed. The challenge was to find a treatment that was effective and would not obscure early evidence of recurrence.

Imiquimod, a topical immunomodulator, was chosen over surgical modalities, along with close follow-up and serial IVCM examinations. Before initiation of treatment, IVCM revealed features consistent with atypical melanocytic proliferation predominantly in the right nasal region (Figure 2B and C). The patient applied imiquimod to his nose and cheek twice a day for 1 week until erythema and irritation occurred, and then once a day for 4 additional weeks. One week after he discontinued the therapy, an IVCM examination demonstrated atypical cells and inflammatory infiltrate on his nose. Two weeks later, another IVCM examination revealed only inflammation and fibrosis (Figure 2D). The results of a skin biopsy subsequently confirmed the absence of atypical melanocytes (Figure 2E). Imiquimod therapy was restarted, with application every 3 days for 6 months to minimize significant erythema and irritant response. At the 1-year follow-up visit, a biopsy specimen and an IVCM examination showed no evidence of recurrence (Figure 2F).

CASE 2

A 55-year-old man presented after skin biopsy specimens revealed an incompletely excised recurrent invasive melanoma on the left site of his forehead. Four years earlier, a nonulcerated, superficial, spreading malignant melanoma, 1.20 mm in thickness (Clark level IV), was completely excised from the site with 1.5-cm margins. A metastatic workup revealed no abnormalities at that time. The patient’s family history was significant in that his mother had a history of 2 malignant melanomas. Three separate areas of macular hyperpigmentation were initially noted. Four biopsy specimens were obtained, including 1 from the normal-appearing skin between the hyperpigmented sites. Histologic analysis revealed 2 malignant melanomas in situ: a 0.7-mm-thick (Clark IV) invasive malignant melanoma and a 1.1-mm-thick (Clark IV) invasive melanoma. Systemic workup showed no evidence of metastasis. On follow-up 4 weeks later, 3 additional brown-gray nonulcerated macules, measuring 2 to 3 mm in diameter, were identified within 1 to 2 cm of the primary malignant melanoma excision site, which was without nodularity or abnormal pigmentation (Figure 3A). Multiple lesions with apparently noncontiguous extension are difficult to evaluate for affected margins. The therapeutic challenge was
to find an effective way to determine the preoperative surgical margins for tissue-sparing purposes.

In vivo confocal microscopy was performed to delineate surgical margins, which could not be distinguished clinically (Figure 3B and D). Therefore, we selected and marked several sites, including the pigmented macules and clinically normal skin between the affected sites and the peripheral sites. The selected areas were imaged and screened for the presence of features suggestive of atypical melanocytic proliferation. The results of skin biopsies corroborated the findings of IVCM. Two weeks later, local wide excision was performed and histologic analysis confirmed a 0.4-mm-thick melanoma and a dermal nodule of melanoma with negative margins (Figure 3C and E). At the 11-month follow-up visit, history and physical examination showed no recurrence.

CASE 3

A 57-year-old man presented to an outside clinic with a 6-month history of a darkly pigmented lesion over his right temple. A shave biopsy specimen revealed a non-ulcerated lentigo maligna melanoma with a desmoplastic neurotropic component at a minimum depth of 0.5 mm, Clark level IV. The patient was referred to the cutaneous oncology clinic, where visual examination showed a light-brown, slightly erythematous 1.5-cm patch with a central 6-mm black-gray macule and poorly defined margins over the right temple (Figure 4A). The lesion had poorly demarcated margins that characteristically include a broad intraepidermal lentiginous component (Figure 4A). The therapeutic challenge was to enhance visualization of the involved sites during tissue-sparing surgical excision. Therefore, IVCM guided the surgical excision (Figure 4B-F). The patient underwent wide local excision 1 cm beyond the positive margins identified by IVCM (Figure 4B). Histologic analysis of the resected specimen showed residual melanoma invasive to a depth of 1.7 mm with clear margins and a lentiginous radial growth phase. No regression or lymphatic or vascular invasion was seen. A subcutaneous node was involved by melanoma, with subsequent sentinel nodes negative for metastatic disease. Metastasis was found in the temporal region of the right side of the patient’s brain, and he underwent 4 months of ra-
diation treatment and adjuvant interferon therapy. He was free of disease 4 months after stereotactic radiosurgery.

**COMMENT**

The extent of atypical melanocytic proliferation is difficult to assess by visual examination alone. These processes commonly have poorly defined borders and may be amelanotic and/or involve lentiginous proliferation, posing an even more difficult challenge to achieve complete excision. They also may mimic other benign dermatologic conditions or skin neoplasms, which can lead to treatment that obscures the diagnosis or histologic analysis. In 2001, Busam et al were the first to describe...
the use of IVCM for the diagnosis of amelanotic malignant melanomas as well as for the delineation of surgical margins. Busam et al also used IVCM to diagnose an intraepidermal malignant melanoma in 2002.

Surgical excision is the current standard of treatment, with surgical margins determined by visual examination. Sober et al published guidelines for the surgical treatment of primary cutaneous melanoma, including specific amounts of clearance around the area of visual abnormality. However, these recommendations may not be applicable for melanocytic proliferations with poorly defined borders, such as lentigo maligna melanoma, amelanotic melanoma, and lesions with lentiginous extension. Recently, novel potential treatments have emerged for the nonsurgical management of melanoma, such as fluorouracil and imiquimod therapy in addition to radiation therapy. Imiquimod is believed to be effective by induction of cytokine synthesis and secretion from epidermal keratinocytes, thereby potentiating immune reactions such as B cell, NK cell, and macrophage activation.11 In 2000, Ahmed and Berth-Jones published the first account of the successful use of the immunomodulator imiquimod as a treatment for lentigo maligna. There are only 3 other reports of its successful use in the treatment of lentigo maligna, including 2 non-peer-reviewed reports.12

Confocal scanning microscopy was invented by Marvin Minsky in 1957. Ten years later, biologic images of brain and ganglion cells using this technique were published.13 It was not until 1990 that the first IVCM images of the human skin using a tandem scanning confocal microscope were reported.14 Rajadhyaksha et al advanced this technique using a low-power laser as light source that improves image contrast and resolution, showing excellent correlation with the results of histologic analysis. Studies since then have continued to show good correlation between characteristics that are appreciable with IVCM and those that are observed by histologic sectioning.2-5,7,10,17-19 However, specific cytologic characteristics cannot be visualized as well with the current technique as with regular histologic examination. Also, amelanotic neoplastic dermal processes are difficult to evaluate by IVCM primarily because of imaging depth limitations and refractivity of inflammatory cells.

In vivo confocal microscopy is used as a noninvasive method of examining the microanatomy of the epidermis and papillary dermis, up to a maximum depth of 350 µm. By spatial filtering, laser scanning confocal microscopy eliminates out-of-focus light, or “flare,” in the specimen. Optical sections are produced by a laser that illuminates the tissue and by scanning one or more focused beams of light across the specimen. Backscattered and reflected light from in-focus planes are detected by the optical system. Contrast is provided by differences in the refractive index of cell organelles and other microstructures, thus allowing visualization of nuclear, cellular, and architectural detail.17 In vivo confocal microscopy imaging preserves the natural architecture of the tissue, including cellular hydration, natural tonicity, and, thus, the natural contrast of structures,17,18 while enabling the assessment of details in the same tissue over time.20-22 With this technique, normal melanocytes, as well as abnormalities such as eccentric nuclei and loss of keratinocytic demarcation, may be discernible on human skin. However, in some instances, it may be difficult to differentiate between melanocytes and pigmented keratinocytes. It is also important to mention that performing margin demarcation with IVCM is time consuming. With the current system, obtaining complete margins for a 1-cm-diameter lesion would require a minimum of 1 hour. In our experience, the time commitment required to perform margin demarcation with IVCM, and obtaining confirmatory skin biopsy specimens, outweighs the morbidity associated with submitting patients to additional surgery as a result of incomplete excisions.

The 3 melanoma cases reported herein illustrate the application of IVCM in diagnosis, in surgical and nonsurgical management, and in the preoperative delineation of surgical margins. As novel treatments emerge and become more available, IVCM can play a significant role in the initial examination, and it may serve as a diagnostic tool by improving sensitivity in diagnosis. It can also increase the rates of successful initial excision and provide a noninvasive means of monitoring response to therapy. However, more studies are needed to prove its efficacy and to further define its clinical applications.

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**News and Notes**

The 11th International Conference on Behçet Disease and Third International Convention for Patients With Silk Road Disease (Behçet Disease) will be held from October 27 to October 31, 2004, in Antalya, Turkey. Further details are available from the Organizing Secretariat, FIGUR Congress and Organization Services, Ayazmadesi Cadd Karadut, sok No. 7, 80888 Dikilitas-Istanbul, Turkey (telephone: 90 (0212) 2586020; fax: 90 (0212) 2586078; e-mail: behcet2004@figur.net; Web site: http://www.bhcet2004.org).