In vivo confocal scanning laser microscopy of pigmented Spitz nevi: Comparison of in vivo confocal images with dermoscopy and routine histopathology

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Background: Spitz nevus is a benign melanocytic lesion sometimes mistakenly diagnosed clinically as melanoma.

Objective: Our aim was to evaluate in vivo reflectance-mode confocal scanning laser microscopy (CSLM) aspects of globular Spitz nevi and to correlate them with those of surface microscopy and histopathology.

Methods: A total of 6 Spitz nevi, with globular aspects on epiluminescence observation, were imaged with CSLM and subsequently excised for histopathologic examination.

Results: A close correlation among CSLM, epiluminescence, and histopathologic aspects was observed. Individual cells, observed in high-resolution confocal images, were similar in shape and dimension to the histopathologic ones. Lesion architecture was described on reconstructed CSLM images. Melanocytic nests corresponded to globular cellular aggregates at confocal microscopy and to globules at epiluminescence observation. Melanophages were clearly identified in the papillary dermis both by confocal microscopy and histopathology.

Conclusion: In vivo CSLM enabled the identification of characteristic cytologic and architectural aspects of Spitz nevi, correlated with histopathology and epiluminescence microscopy observation. (J Am Acad Dermatol 2004;51:371-6.)

The epithelioid and/or spindle cell nevus, also called “Spitz nevus,” is an acquired, usually benign, melanocytic tumor. Its alarming clinical presentation may sometimes lead to its being mistakenly diagnosed as melanoma. Using epiluminescence microscopy (ELM), characteristic features of different types of pigmented skin lesions have been identified.1-3 In such instances, ELM has been shown to improve diagnostic accuracy of Spitz nevi,4-7 in particular when typical findings, such as the globular aspect and/or the presence of a rim of large globules, are present. Because the differentiation between Spitz nevus and melanoma may be sometimes very difficult, histopathology is usually performed for the definitive diagnosis. Typical histopathologic aspects, which help the pathologist to distinguish between Spitz nevi and melanomas, have been identified8-11 as architectural patterns and cytologic features.12

In vivo reflectance-mode confocal scanning laser microscopy (CSLM) is a novel technique providing instantaneous visualization of skin structures at a cellular-level resolution.13,14 Its recent application in experimental dermatology for the characterization of melanocytic lesions enabled the identification of specific features associated with melanoma, and dysplastic and benign nevi, tightly correlated with conventional histopathologic examination.15-19

The aim of this study was to evaluate in vivo CSLM aspects of globular Spitz nevi or Spitz nevi with peripheral globules. The implementation of software for image reconstruction of CSLM pictures enabled

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the description of the architecture of the lesion. Together with cytologic features, the former was used to establish a correlation between ELM images and histopathologic sections.

MATERIALS AND METHODS

Patients

This study included 6 Spitz nevi in as many patients, with an average patient age of 29.8 years. Before biopsy, all lesions were recorded using both ELM and CSLM. To have an exact correspondence between ELM and CSLM images, the CSLM adapter ring was first positioned onto the skin and centered around the lesion. Subsequently, ELM and CSLM images were acquired. All lesions were then excised and underwent histopathologic examination for diagnostic confirmation.

Evaluation methods

ELM imaging was performed with a digital videomicroscope (VMS-110A, Scalar Mitsubishi, Tama-shi, Tokyo, Japan) using 20-, 50-, and 200-fold magnification, positioning the probe onto the CSLM adapter ring. The instrument has been described elsewhere.20 The images were digitized by means of a Matrox Orion frameboard (Matrox Electronic Systems, Ltd, Dorval, Quebec, Canada) and stored by an image acquisition program (VideoCap 8.09, DS-Medica, Milan, Italy), which runs under Microsoft Windows (Microsoft Corp., Redmond, Wash). The digitized images offer a spatial resolution of 768 × 576 pixels and a resolution of 16 million colors.

For CSLM imaging, confocal images were acquired by means of a near-infrared reflectance confocal laser scanning microscope (Vivascope 1000, Lucid Inc, Henrietta, NY).14 After acquiring the ELM image, the adapter ring was filled with water and the arm of the CSLM with the 30x water immersion objective lens (numeric aperture of 0.9) was placed onto it. The instrument uses a diode laser at 830 nm with a maximum power of 35 mW at tissue level, enabling visualization of skin structures at a maximum depth of 350 μm. Images have a spatial resolution of 0.5 to 1.0 μm in the lateral dimension and 4 to 5 μm in the axial dimension. Each image corresponds to a horizontal plane of skin section at a selected depth with an effective field of view of 475 × 350 μm, with a resolution of 640 × 480 pixels and 255 colors. An automated stepper was used to obtain a grid of 16 contiguous horizontal images at a selected depth, constructing a montage image with an in vivo field of view of 1.9 × 1.4 mm (block image). A sequence of 30 block images was acquired for each lesion at dermoepidermal junction level and mounted by means of a software developed by us to obtain a field of view of 7.60 × 6.65 mm (reconstructed image) (Fig 1). Sequences of confocal sections, beginning at the stratum corneum and into the papillary dermis, were recorded at areas of interest on the border and inside the lesion.

Image description

For ELM images, traditional dermoscopic features were described for each lesion.21

CSLM images of Spitz nevi were described considering both the overall aspect of the lesion, evaluated on the reconstructed image with a field of 7.60 × 6.65 mm, and the cytologic features, evaluated on the single images with the best resolution (475 × 350 μm). For the overall aspect, the symmetry, border cutoff, and cell and structure uniformity were taken into account. According to previous reports concerning normal skin or melanocytic lesions observed with CSLM, cytologic features were evaluated at different depth. The standard confocal features of the epidermis and of the dermis were described.14-18 Moreover, the presence within the superficial layers of ovoid highly refractive structures without nuclei was reported. At the basal layer, cells clustered into globular aggregates were described also considering their size, shape, brightness, and homogeneity throughout the lesion. The cell dimension was compared with keratinocytes of the surrounding healthy skin.

Fig 1. Reconstructed confocal scanning microscopy image of Spitz nevus, used for evaluation of architectural features. Lesion appears more refractive in comparison with surrounding skin. Inset, Corresponding epiluminescence image as observed with polarized light and 50-fold magnification.
The traditional histopathologic description of both architectural patterns—such as lesion symmetry; border cutoff; aspect and distribution of the nests; epidermal hyperplasia and flogistic infiltrate; and cytologic features, such as cell type and aspect, presence of pagetoid infiltration, number of mitoses, cell maturation with increasing depth, and cell uniformity from one side of the lesion to the other—were described.

RESULTS

ELM features

All the 6 Spitz nevi were symmetric lesions with a diameter ranging between 4 to 8 mm, characterized by a globular appearance. In 5 cases a rim of large globules was present at the periphery of the lesion. The central portion was characterized by darkly pigmented globules in 3 cases and light brown in the remaining 3, associated with gray-bluish areas in 4 lesions.

CSLM ASPECTS

Evaluation of lesion architecture on the reconstructed image. As shown in Table I, Spitz nevi markedly differed from the control skin. Melanin present in nevus cells represented a strong source of contrast, rendering the pigmented skin lesion lighter than the surrounding skin (Fig 1). All lesions presented a symmetric silhouette, with cell and structure uniformity. Sharp borders were observed in 5 Spitz nevi with the globular rim.

Evaluation at cellular level. The normal honeycombed pattern, constituted by polygonal low refractive cells with a mean diameter of 22 \( \mu \text{m} \), was always observable in superficial layers, whereas bright refractive particles were noticed in 4 out of 6 lesions. Moreover, few individual cells, round in shape and with bright cytoplasm and dark nucleus, with a mean diameter of 35 \( \mu \text{m} \), were observed in one lesion. In two cases ovoid highly refractive structures with a diameter of approximately 50 \( \mu \text{m} \) were observed at the basal layer or immediately upward (Fig 2). Small bright cells were found at the dermoepidermal junction, correlating with melanocytes and pigmented keratinocytes seen by conventional histopathology, frequently with intercalated large brighter cells that were round to oval (all cases) and spindle or dendritic (4 cases) in shape (Fig 3). Oval to round polygonal aggregates with well-defined borders, composed by clustered cells, frequently large in size and highly refractive, were observed in all lesions, corresponding in structure and dimension to the pigmented globules of the ELM observation and to melanocytic nests at the histopathologic examination (Fig 4). All lesions examined presented contiguous dense nonconfluent aggregates, with variable diameter ranging between 140 to 420 \( \mu \text{m} \), homogeneously distributed inside the lesion. Well-demarcated, highly refractive aggregates formed by large homogeneous cells were observed at the periphery of the 5 Spitz nevi with a globular rim. Plump, bright cells with ill-defined borders, corresponding to melanophages, were present in different amounts in all lesions (Fig 3, C). In 4 cases a great amount of plump cells was observed, correlating with the presence of ELM gray-bluish areas and of a great histopathologic inflammatory infiltrate rich in melanophages. Small canalicular structures within the papillary dermis, corresponding to capillaries, were evident in all cases.

Histopathologic examination. All lesions were symmetric and well circumscribed. Epidermal hyperplasia with elongated rete ridges was observed only in one case. Usually large nests, homogeneous in size, predominantly at the dermoepidermal junction, were observed in all Spitz nevi. No permeation of the epidermis was reported. Although a flogistic infiltrate was present in all lesions, it was marked and

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rich in melanophages in 4 out of 6 cases. Cytologic features consisted predominantly of large epithelioid cells, intensely pigmented in 4 cases. Poorly pigmented spindle cells predominated only in one case. Cell uniformity and maturation was observed in all cases. Transepidermal melanin loss was reported in 4 lesions. In two cases, large coarse pigment conglomerates inside the epidermis were present.

**DISCUSSION**

In vivo reflectance-mode CSLM is a novel technique that enables the in vivo study of the skin at...
a nearly histopathologic resolution, producing pictures of horizontal planes of the skin. Confocal images of normal skin and of keratinocytic and inflammatory skin lesions with detailed correlation to histopathologic sections have been previously reported. Because melanin represents a strong source of contrast, the use of this technique for the characterization of pigmented skin lesions seems particularly interesting. The appearance of melanocytes, pigmented keratinocytes, and melanophages, together with the features of common and atypical nevi, and of melanomas, have been described. Because dermoscopy enables the visualization of subsurface structures that can be correlated with specific histopathologic aspects, the capability of CSLM in identifying cytologic and architectural features with a tight correlation to histopathology may improve diagnostic accuracy, especially for pigmented skin lesions characterized by unspicific features, such as in situ melanoma versus atypical nevus or lentigo maligna versus lentigo maligna melanoma.

Spitz nevi may represent a diagnostic pitfall both for dermatologists and for pathologists. Histopathologic criteria useful for distinction between melanomas and Spitz nevi have been identified and defined. ELM description of Spitz nevi enables the subdivision of these lesions into 3 main categories: globular; starburst; and atypical. The aim of our study was to describe Spitz nevi of the globular type by CSLM. With specific software for image reconstruction of CSLM images, we were able to describe the overall aspect and the architecture of Spitz nevi. This approach also enabled the exact correlation with the corresponding ELM and histopathologic features. On the whole, confocal images of our globular Spitz nevi were characterized by aggregated globules. They were uniform in size and shape, and tended not to become confluent, in accordance to histopathology. All Spitz nevi with peripheral globules presented refractive polygonal aggregates at the periphery, corresponding to melanocytic nests at the margin of lesion in the histopathologic sections. In confocal images, superficial layers presented a normal honeycombed appearance. In the 4 Spitz nevi characterized by dark pigmentation, bright granular particles between the meshes of the honeycombed pattern were observable, probably corresponding to transepidermal melanin loss at the histopathologic examination. Few individual cells, round to oval in shape, with bright granular cytoplasm and dark eccentric nucleus, were observed in the suprabasal layers in one case, corresponding to pagetoid infiltration of nevomelanocytic cells. Although detection of the pagetoid spread of melanocytes within the epidermis is an important clue for melanoma diagnosis, this feature is also observable in Spitz nevi, which are usually characterized by the presence of sporadic cells only in suprabasal layers. Moreover, the presence in two Spitz nevi of ovoid homogeneously bright structures in basal and suprabasal layers appeared correlated with large coarse pigment conglomerates inside the epidermis at the histopathology (Fig 2).

In conclusion, in globular Spitz nevi, CSLM allowed the in vivo recognition of characteristic histopathologic aspects, with an excellent correlation with the corresponding ELM features. The implementation of a program for the composition of an overall CSLM image enabled the evaluation of the architectural aspects of the lesions and the correlation of ELM features with the exactly corresponding confocal structures. Single cells or cellular aggregates can be imaged for the cytologic description of the lesion and for the correlation with histopathology. Thus, CSLM may represent the missing link between the ELM technique and the histopathologic examination.

REFERENCES


