Reflectance Confocal Microscopy of the Yellow Dot Pattern in Alopecia Areata

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Background: The presence of yellow dots is a characteristic dermoscopic finding in alopecia areata. The aim of this study was to investigate the yellow dot pattern observed at dermoscopy in alopecia areata with reflectance confocal microscopy (RCM) and correlate RCM findings with pathological features.

Observations: Six patients affected by alopecia totalis entered the study. Patients were first submitted to scalp dermoscopy, which was followed by RCM examination of the same area. After RCM, a 5-mm punch biopsy specimen was also taken. Dermoscopic findings showed the yellow dot pattern in all patients, with round or polycyclic yellow-pink dots often containing miniaturized or broken hair shafts. At RCM, a Vivablock mosaic taken at the level of the spinous layer showed striking reduction of follicular adnexal structures and empty lumina containing highly refractile material corresponding to the yellow dots seen on dermoscopy. The pathological features showed that the yellow dots correspond to the dilated infundibula of the velluslike anagen and telogen follicles that characterize the chronic phase of alopecia areata.

Conclusion: The RCM study of the yellow dot pattern showed a good correlation with the dermoscopic and pathological findings and confirms that the yellow dots correspond to inefficient follicular structures that often contain hair remnants.

Arch Dermatol. 2011;147(1):61-64

In vivo reflectance confocal microscopy (RCM) is a noninvasive technique for real-time en face imaging of the superficial layers of the skin down to the superficial reticular dermis, with cellular-level resolution close to conventional histopathological analysis.1 Contrast is provided by differences in the refractive index and size of different cellular organelles as well as the extracellular microstructures within the tissue.2,3 Reflectance confocal microscopy has been used for the evaluation of several inflammatory skin conditions, such as acute contact dermatitis,4 discoid lupus erythematosus,7 and psoriasis,8 and has been correlated with conventional histologic analysis in several instances.3,6

Alopecia areata is a common form of a noncicatricial alopecia, characterized by patchy hair loss in the absence of inflammatory signs. In the last few years, dermoscopy has been increasingly used in the evaluation of patients with hair loss. This new diagnostic method has provided new clinical signs for recognizing hair diseases and enhanced features previously seen with the naked eye.9 The presence of yellow dots is a characteristic even though not a specific finding in alopecia areata. This pattern is characterized by a distinctive array of yellow to yellow-red, round and polycyclic dots that vary in size and possibly correspond to keratin and sebaceous debris contained within the dilated ostium of anagen and miniaturized hair-containing follicles.10 The aim of this study was to examine the yellow dot pattern with RCM and correlate RCM findings with dermoscopic and pathological features.

Methods

The ethics committee of the IFO Institute, Rome, Italy, approved this study. Six white patients (4 men and 2 women), aged 18 to 49 years (mean, 32.6 years) signed an informed consent form and entered the study. All patients were affected by alopecia totalis; mean disease duration was 8 years. Patients first underwent scalp dermoscopy, which was followed by RCM examination of the same area. After RCM, a 5-mm punch biopsy specimen for histological examination was obtained.

Dermoscopic images were obtained through computerized polarized light videomicr-
copy (FotoFinderdermoscope; Teachscreen Software, Bad Birnbach, Germany), with lenses with magnification factors of ×20 to ×70 at ×10 increments. Epiluminescent mode was used with thermal water as the immersion fluid.

We used a commercially available Vivascope 1500 RCM device (Lucid Technologies, Henrietta, New York) for clinical in vivo imaging. This system includes a diode class 3A Laser (European version) with a wavelength excitation maximum at 830 nm and power lower than 35 mW at tissue level. A 30 × 0.9-NA (numerical aperture) water immersion objective lens was used. The RCM was attached to the skin using an adhesive ring to reduce motion artifacts during examination. Immersion media included water between the adhesive window and the skin and ultrasound gel between the adhesive window and the objective lens. Details of this system have been reported previously.11 Single images (0.5 × 0.5 mm) were obtained from different skin levels for descriptive analysis. In addition, Vivablock software (Lucid Technologies) was used to obtain mosaics of 16 to 64 images (2 × 2 to 5 × 5 mm) at the level of the spinous layer. These mosaics were used for correlation with dermoscopy to define adnexal structure distribution, dimension, and density and for yellow dot location. Vertical VivaStack software imaging (Lucid Technologies) was used to evaluate the adnexal structures from the ostium to the reachable dermis. Stacked images were captured starting from the squamous layer and progressing deeper in 5-µm steps until reaching the last visible dermis.

RESULTS

DERMOSCOPY

Dermoscopy at low magnification revealed the yellow dot pattern in all patients. Yellow dots appear as round or polycyclic, yellow to yellow-pink dots, which may be devoid of hair or contain miniaturized short regrowing hairs, cadaverized hairs, or dystrophic hairs (Figure 1).

REFLECTANCE CONFOCAL MICROSCOPY

Vivablock mosaic images taken at the level of the spinous layer showed a striking reduction of follicular adnexal structures and the presence of empty adnexal lumina fulfilled by highly refractile material corresponding to yellow dots seen on dermoscopy (Figure 2 and Figure 3). Some lumina occasionally contained remnant of hair structures (Figure 4). The VivaStack software study of the yellow dots revealed highly bright luminal structures extending from the stratum corneum to the upper dermis. No or few inflammatory cells were seen.

PATHOLOGICAL ANALYSIS

Specimens from 5-mm punch biopsies obtained from the scalp region exhibiting the yellow dot pattern showed active alopecia areata lesions. Horizontal sections at the upper level revealed widened infundibula containing keratinous debris and occasionally hair shaft fragments, bacteria, and yeasts (Figure 5). Sections at the isthmus...
level showed an increased number of velluslike anagen and telogen follicles, catagen follicles, and telogen germinal units surrounded by a mild lymphocytic infiltrate. Sections at the bulbar level showed a perifollicular and lymphocytic infiltrate with the typical “swarm of bee” pattern diagnostic for alopecia areata.

**COMMENT**

At RCM examination of alopecia areata, yellow dots are clearly visible during the evaluation of the spinous layer as highly refractile, round structures brighter than duct glands. Deeper into the skin (using RCM and Vivastack software evaluation) at the upper dermis level, the yellow dots clearly disclose their correspondence to small follicles, in which the epithelial component, surrounding a homogeneously bright central, round area, is clearly visible. The epithelial component is smaller than in a normal hair follicle, and a homogeneous material presenting a reflectance index similar to keratin composes the central bright area. Furthermore, in some yellow dots, remnant hair structures can also be seen, confirming the correspondence between yellow dots and inefficient and damaged follicular structures. Findings from the pathological study showed that the yellow dots correspond to the dilated infundibula of the velluslike anagen (also referred to as “nanogen”) and telogen follicles that characterize the chronic phase of alopecia areata.12 RCM tech-

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**Figure 3.** Luminal structures surrounded by follicular epithelium and filled by highly bright material corresponding to keratin (red arrow). Black lumina with epithelial structures corresponding to duct glands (green arrow). Inflammatory cells are present around adnexal structures (white arrows).

**Figure 4.** Highly bright material inside a luminal structure seen at the level of the spinous layer and corresponding to the yellow dot.

**Figure 5.** Pathological analysis. A, Horizontal section at infundibulum level showing dilated infundibula containing keratin material and bacteria. Note the presence of mild lymphocytic infiltrate in the dermis (hematoxylin-eosin, original magnification ×4). B, Higher magnification showing a dilated infundibulum filled with keratin and hair shaft debris (hematoxylin-eosin, original magnification ×40).
nology can be used to evaluate the presence of inflammatory cells with adnexotropism and sclerosis of the upper dermis features that are generally absent in alopecia areata.

Our study shows that RCM technology permits non-invasive evaluation of the upper follicle with very good correlation with pathological analysis. General limitations of the technique include the high cost of the device, the time required to evaluate large skin or scalp areas, and the necessity of specific training with the technology before its routine application. An important limitation of RCM in the study of hair disorders is the loss of image resolution at the level of the upper dermis, which makes the technique not useful in the study of the lower follicle.

Further studies are needed to define if RCM can also provide information on yellow dots seen in other conditions such as alopecia areata incognita or advanced androgenetic alopecia or prognostic data in patients with alopecia areata.

Accepted for Publication: December 22, 2009.

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Author Contributions: Drs Ardigò and Tosti had full access to all of the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis. Study concept and design: Ardigò, Tosti, Cameli, and Vincenzi. Acquisition of data: Ardigò, Tosti, Cameli, Vincenzi, and Misciali. Analysis and interpretation of data: Ardigò, Tosti, Cameli, Vincenzi, and Berardesca. Drafting of the manuscript: Ardigò, Tosti, Cameli, Vincenzi, and Berardesca. Critical revision of the manuscript for important intellectual content: Ardigò and Tosti.

Administrative, technical, and material support: Ardigò, Tosti, Cameli, Vincenzi, Misciali, and Berardesca. Study supervision: Ardigò and Tosti.

Financial Disclosure: None reported.

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