Reflectance Confocal Microscopy: An Effective Diagnostic Tool for Dermatophytic Infections

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Practice Points

- Current methods for diagnosing dermatophytosis can be invasive, with variable sensitivity and/or slow turnaround time.
- Reflectance confocal microscopy is a promising option for rapid noninvasive diagnosis of dermatophytosis.

Current methods for diagnosing dermatophytic infections have various drawbacks. Analysis via skin scrapings and biopsies can be invasive and/or take too long to yield results. Reflectance confocal microscopy (RCM) is an emerging in vivo imaging technology that can potentially be used to diagnose cutaneous dermatophytic infections. This modality provides high-resolution images of the skin extending to the level of the superficial reticular dermis that could reveal the presence of fungal hyphae. In this retrospective chart review, we investigated the application of RCM as a diagnostic tool in the setting of a private practice. Images were used to diagnose dermatophyte infections and the results were compared to those of other established diagnostic methods. We found RCM to be a potentially effective and highly sensitive tool in the diagnosis of cutaneous dermatophytic infections.

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There are a variety of well-established methods for diagnosing dermatophyte infections, including potassium hydroxide (KOH) preparations, fungal cultures, and skin biopsies. Each modality has its place in clinical practice, but they also have drawbacks. Reflectance confocal microscopy (RCM) is an emerging in vivo technology that could potentially serve as a sensitive, rapid, and noninvasive method of diagnosing dermatophytosis. Using near-infrared laser light scanning, RCM provides a quick noninvasive method of generating black-and-white, horizontal, quasipathology images that allow for the identification of cells and other structures similar to dermoscopy and histopathology.[1] The images are obtained in a fully noninvasive fashion, as the device is placed in contact with the skin using a liquid medium. The process takes 5 to 15 minutes depending on the number of images obtained, and the images can then be displayed in real time on a computer screen or transmitted to a pathologist for evaluation.

Most initial applications of RCM focused on evaluating melanocytic lesions with the primary goal of differentiating between benign nevi and melanomas, thus reducing the need for skin biopsies.[2-4] Efforts to develop RCM diagnostic criteria for identification of other skin cancers[5,6] as well as to aid in the diagnosis of nonneoplastic skin conditions are ongoing.[7] The potential applications of RCM are virtually limitless, as this modality can (at least partially) take the place of biopsies in a variety of clinical scenarios.[2,8] Few reports have documented the utility of RCM

as a diagnostic tool for onychomycosis[9,10] and dermatophytic infections of the skin.[11,12] Hui et al[13] reported use for RCM for microscopic evaluation of mycelium features. Turan et al[14] found that RCM could not replace the current diagnostic standards for tinea incognito but may be successfully used as an in vivo noninvasive screening tool to facilitate diagnosis. Because it provides high-resolution horizontal images extending from the surface of the stratum corneum to the superficial reticular dermis, RCM could be an effective tool in the diagnosis of cutaneous dermatophyte infections, as organisms usually are located in the stratum corneum of the epidermis in this infection. Branching hyphae are readily visible in the stratum corneum on RCM (Figure).

We reviewed a series of 9 cases from a private practice setting in which RCM was used to diagnose dermatophytosis. We compared the diagnostic accuracy of RCM to results from other diagnostic methods and the ultimate clinical outcome to determine the usefulness of this new technology.

Methods

Our retrospective chart review included all cases in which RCM was used and the clinical differential diagnosis included tinea corporis over a 4-month period in a private, single-specialty dermatology practice. All patients were treated by the same dermatologist. The RCM images were taken using an imaging system that had a horizontal optical resolution of less than 1.25 μm and a vertical optical resolution of less than 5.0 μm. The imaging was performed by medical assistants who were trained by the device manufacturer.

The sample sites were cleaned with isopropyl alcohol and a translucent contact ring was affixed to the skin using a liquid medium. The imaging head of the device was connected to the imaging ring and the images were taken. Identical imaging protocol was used in all patients. Multiple sets of horizontal images and one stack of vertical images were obtained. Patients reported no discomfort during the procedure, and the entire process was usually completed within 15 minutes. The images were sent to the pathologist for evaluation using the manufacturer's telepathology system and were returned with a diagnosis within 24 hours. (On-site, real-time diagnosis also is possible if the dermatologist is trained in interpreting the images.)

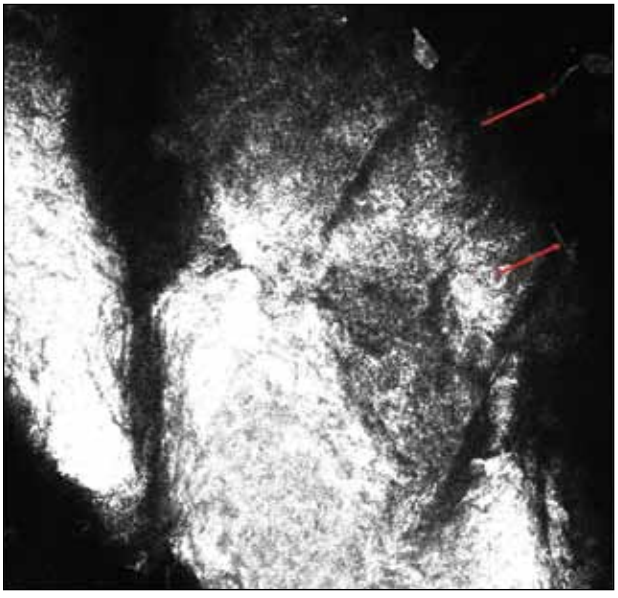
In the chart review we looked for other diagnostic methods used as well as clinical outcomes. A case was considered to be positive for dermatophytic infection if any of the other diagnostic modalities yielded positive results or if a definitive resolution of the condition could be achieved using antifungal treatments alone.

Results

Ten patients (mean age, 43.1 years; age range, 16–76 years) with lesions that presented as possible dermatophytic infections underwent RCM analysis. In addition to RCM imaging, 5 patients underwent KOH testing of skin scrapings, 3 underwent analysis by fungal culture utilizing dermatophyte test medium (DTM), and 5 underwent biopsies. The findings are further summarized in the Table. One patient (patient 5) was excluded from the study because the RCM could not be evaluated due to the poor quality of the confocal images. Additionally, 2 patients (patients 2 and 7) had suboptimal imaging, which limited the evaluation.

Of the 9 evaluable cases, 4 (patients 1–4) were determined to be positive for the presence of dermatophytic infection through the fulfillment of criteria independent of RCM imaging. In each of those 4 cases, RCM images revealed the presence of hyphae, which indicated the presence of dermatophytic infection. In these 4 cases, RCM and other diagnostic methods reached the same diagnosis.

In the other 5 cases (patients 6–10), the final diagnosis was not a dermatophytic infection. In 4 of those cases (patients 7–10), there were no signs of any structure resembling hyphae on the RCM images; however, in 1 case (patient 6), the RCM images showed structures that were consistent with the appearance of hyphae to the extent that the investigators, based solely on analysis of the RCM images, deemed a diagnosis indicating presence



Branching hyphae (red arrows) seen in the epidermis on reflectance confocal microscopy.

Overview of Findings[a]

Patient No.	Age, y	Diagnosis of Tinea	Patient History	Description of Lesion(s)	RCM Findings	KOH Results	DTM Results	PAS Stain of Biopsy	Skin Biopsy Results	Treatment Outcome
1	29	+	9 mo itchy rash on back	Scaly, red, oval plaques on back	Branching hyphae	Branching hyphae	N/A	N/A	N/A	Resolved with econazole
2	16	+	4 d rash on neck	Scaly, red, oval plaque on right neck	Suboptimal image quality; few lesions were suspicious for hyphae	Branching hyphae	N/A	N/A	N/A	N/A
3	46	+	3 y recurrent rashes on legs, hands, back	Scaly, red, oval plaque on right leg	Branching hyphae	N/A	–	N/A	N/A	Resolved with econazole
4	25	+	4 mo rashes on trunk	Scaly, red, 3–6-cm, oval or annular plaques on trunk	Branching hyphae	Branching hyphae	N/A	N/A	N/A	Improved with econazole and cleared with systemic terbinafine
5[b]	47	+	4 wk rash on left leg	15-cm red, annular, scaly plaque on left calf	Poor image quality; could not be evaluated	–	N/A	N/A	N/A	Cleared with econazole and systemic terbinafine
6	38	–	5 mo rash on back	Scaly confluent patches on back	Probably tinea; lesions were suggestive of hyphae	–	–	–	Hyperkeratosis and minimal spongiosis	Unresponsive to topical antifungals and steroids; resolved with urea cream 40%
7	28	–	2 wk patches on trunk	Scaly, red, 1–2-cm, oval plaques on trunk	Suboptimal image quality; filamentous structures of unknown etiology	N/A	N/A	N/A	Spongiotic dermatitis, possible pityriasis rosea	Resolved with topical steroids

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Patient No.	Age, y	Diagnosis of Tinea	Patient History	Description of Lesion(s)	RCM Findings	KOH Results	DTM Results	PAS Stain of Biopsy	Skin Biopsy Results	Treatment Outcome
8	54	–	6 mo rash on groin folds, axillae	Dry red patches in the groin folds and axillae	No fungal elements were noted	N/A	N/A	–	Spongiotic dermatitis	Resolved with topical steroids
9	72	–	3 wk rash on right leg	Red, 15-cm, oval plaque on right leg	No fungal elements were noted	N/A	–	N/A	Spongiotic dermatitis	Treated with topical steroids; patient was lost to follow-up
10	76	–	3 wk rash on upper trunk	Dry, red, 5–8-cm plaques on right upper chest and back	No fungal elements were noted	N/A	N/A	N/A	Spongiotic dermatitis	Treatment with topical steroids; patient was lost to follow-up

Abbreviations: RCM, reflectance confocal microscopy; KOH, potassium hydroxide; DTM, dermatophyte test medium; PAS, periodic acid–Schiff; N/A, not applicable.

[a]+ indicates positive; −, negative.

[b]This patient was excluded from the study because the RCM could not be evaluated due to the poor quality of the confocal images.

of a dermatophytic infection to be valid. In this case, a 38-year-old man presented with extensive scaly patches on the back of several months' duration. Repeated skin biopsies showed hyperkeratosis and occasionally minimal spongiosis, while periodic acid–Schiff staining did not reveal fungal elements. Fungal cultures and KOH preparations were negative. Prior treatments with topical antifungals and steroids failed to improve the condition, which resolved rapidly with urea cream 40%. The interpretation of the RCM images in this patient did not match up with the results obtained from other methods of diagnosis and the clinical outcome; thus, we classified it as an incorrect diagnosis based on RCM analysis alone. In total, successful diagnosis using RCM imaging was achieved in 8 of 9 cases included in the analysis.

Comment

In this chart review, we evaluated the utility of using RCM in the diagnosis of dermatophytic infections of the skin by comparing findings noted on confocal imaging with those of other methods of diagnosis (Table). We included cases in which the clinical presentation raised the possibility of dermatophytic infection. Cases were considered positive for dermatophytes if KOH preparation, fungal culture, or skin biopsy (with or without periodic acid–Schiff staining) were positive or if there was a complete response to antifungal treatment alone. In this small number of cases, we found that RCM was 100% sensitive, as hyphae were readily seen in all cases of dermatophytic infections. In 1 RCM-positive case (patient 3), fungal culture with DTM was negative, but antifungal therapy was nonetheless given. Because the lesion resolved promptly with econazole, RCM proved to be true positive and DTM proved to be false negative (Table). Reflectance confocal microscopy imaging, however, was less specific. Of the 5 cases that showed no presence of dermatophytic infection, there was 1 case (patient 6) in which the pathologist could recognize structures that resembled fungal hyphae. There are various possible sources of structures masquerading as dermatophytes on confocal imaging,

including the edges of nonnucleated loose keratinocytes, keratin fragments, and other foreign fibers. Evaluation by an experienced investigator can certainly help in limiting false-positive analyses, but a larger case study would be useful to develop a set of specific criteria to aid in the differentiation of fungal hyphae from other artifacts as well as to further define the sensitivity and specificity of RCM.

We also encountered difficulties with the technical aspects of RCM. One case (patient 5) was excluded from the analysis because the images were poor quality and could not be interpreted, and 2 cases (patients 2 and 7) had suboptimal images, in part due to operator error and in part due to equipment error that was recognized later on. The technical difficulties were problematic because no immediate review of image quality was available while patients were still present for possible reimaging. All of the images evaluated in this study were captured shortly after the RCM device was introduced to the practice. It is possible that with more training and a quick, on-site review of image quality, these technical problems could be avoided. Imaging protocols (ie, numbers and levels of scans taken by the confocal microscope) also could be adjusted so they include a large enough range to compensate for potential operator errors; however, these adjustments also could increase overall imaging time.

Conclusion

Based on our chart review of a small number of cases, we found that RCM can be a useful tool in diagnosing dermatophytic infections of the skin. With adequate training, dermatologists may be able to use RCM as an in-office tool to capture and evaluate images and subsequently diagnose or exclude dermatophytosis in a quick and noninvasive manner. However, further research and controlled studies of more cases will be required to develop accurate criteria for diagnosing fungal structures by RCM as well as to help determine the role of RCM in our diagnostic armamentarium.

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