Office Practice–Based Confirmation of Onychomycosis

A US Nationwide Prospective Survey

Boni E. Elewski, MD; James Leyden, MD; Michael G. Rinaldi, PhD; Ercem Atillasoy, MD

Background: Onychomycosis is sufficiently prevalent to be seen and treated by primary care physicians. The diagnosis of onychomycosis is most often confirmed from nail specimens by microscopy and fungal culture done at a central laboratory; these are relatively expensive tests with a turnaround time of a month or more. This study was conducted (1) to evaluate the use of in-office dermatophyte test medium (DTM) culture, and (2) to determine the epidemiology of onychomycosis in a large, nationwide sample of patients who were not participants in a clinical trial.

Methods: A nationwide sample of primary care physicians and podiatrists enrolled 670 patients with clinical signs of toenail onychomycosis. Dermatophyte test medium cultures were performed in the office and the results were compared with fungal cultures performed by a central laboratory.

Results: Central laboratory fungal cultures were positive in 44% (n = 297) of patients and DTM cultures in 51% (n = 345). Dermatophytes accounted for 93% of the confirmed infections and nondermatophyte molds the rest. In the 617 patients with paired dermatophyte test medium and laboratory fungal culture results, the 2 tests were in agreement (both positive or both negative) in 68% of cases (κ, 0.37; asymptotic SE, 0.04; 95% confidence interval, 0.299-0.441).

Conclusions: A DTM culture is a relatively rapid, easy, and inexpensive method to confirm dermatophyte infections in patients with signs of onychomycosis in the primary care setting. Because the available drugs for treating onychomycosis are effective against all dermatophyte species, the confirmation of dermatophyte infection, without further identification of genus and species, is sufficient evidence to begin treatment.
Chromomycosis. Candida infections account for a very small proportion of onychomycosis cases and are not discussed in this article.

Traditionally, in dermatologic practice, the diagnosis of onychomycosis is confirmed by direct microscopic examination of a specimen prepared with potassium hydroxide (KOH) to detect fungal elements and mycologic culture in a central laboratory, and to identify the specific pathogen and confirm that it is viable. The KOH test can indicate the presence or absence of fungal elements. It does not give information as to the viability or etiology of any fungal elements detected. These techniques are specific, but their sensitivity is unknown, with reported culture recovery rates from nail specimens averaging about 50%. Also, results of laboratory fungal culture are usually not available for 4 to 6 weeks. The results of KOH testing are useful to the primary care physician, but the preparation of nail specimens is technically difficult because of the quantity of keratin that it contains, and the interpretation of the results requires experience to be done correctly. Consequently, it is generally better to submit a specimen for testing rather than perform it in the office practice. A KOH result indicating septate hyphae together with a clinical diagnosis of onychomycosis may frequently be used to start treatment because of the high likelihood that the infecting pathogen is a dermatophyte, but the disadvantages of the standard diagnostic tests may be sufficient to dissuade clinicians from obtaining them before treating. On the other hand, reliance on diagnostic methods with limited sensitivity could lead to undertreatment of onychomycosis.

A rapid, easily performed, accurate, low-cost confirmatory test is needed. The present study was conducted to evaluate one such method—the dermatophyte test medium (DTM) culture. The culture medium was originally described by Taplin et al as a test for the presence of dermatophytic molds. A DTM culture is less expensive than a fungal culture at a central laboratory, and results are available much sooner, usually within 3 to 7 days. Dermatophyte growth is indicated by a change in the color of the DTM, from yellow to red in response to alkaline metabolites that result from growth of dermatophytes. The majority of DTM cultures can be identified within 1 week, and fewer than 2% of cultures require 2 weeks to show a change in color. The DTM contains gentamicin and clotrimazole to inhibit bacterial growth and cycloheximide to inhibit growth of saprophytic fungi. Although it does not identify specific organisms, a positive DTM culture confirms the presence of dermatophyte pathogens, which account for the vast majority of cases of onychomycosis. Taplin et al correctly identified dermatophytes by DTM color change alone in 97% of 1400 fungal cultures evaluated. Dermatophyte test medium culture systems are commercially available that appear suitable for use in the general-practice office setting. A DTM culture together with KOH evaluation would be expected to provide accurate and timely guidance for the treatment of onychomycosis. Given the potential benefits of DTM culture to confirm a clinical diagnosis of onychomycosis, the test is underused and may be viewed as inferior to more formal laboratory fungal culture methods such as Sabouraud dextrose agar.

The primary aim of the present study was to compare the sensitivity of office-based DTM culture in the hands of primary care physicians and podiatrists to that of fungal culture in a central mycology laboratory. Because this study included a large patient population distributed throughout the United States, a secondary aim was investigation of the frequency of different causative pathogens and their regional distribution, and clinical patterns of infection. To our knowledge, this is the largest prospective epidemiological survey conducted in patients presenting with signs and symptoms of onychomycosis. The study population may well be more representative than patients treated in clinical trials with restrictive inclusion and exclusion criteria.

SUBJECTS AND STUDY DESIGN

A total of 149 US office-based and clinic-based primary care physicians and podiatrists were recruited for this study. The sample was stratified by specialty to ensure approximately equal representation of primary care physicians and podiatrists, and to represent proportionally the geographic regions of the United States. Each physician was asked to enroll 5 or more patients, aged 18 years or older, with signs and symptoms of onychomycosis. Patients were excluded from the study if they had received oral antifungal therapy within the previous 90 days and any topical antifungal agent within the previous 30 days. Patient enrollment began on July 1, 2000, and data collection was completed May 5, 2001.

At the initial office visit, the primary care or podiatric physician explained the nature of the study, obtained written informed consent, collected demographic information and a relevant medical history, and obtained a specimen from the toenail bed for mycologic evaluation. Specimens were divided and DTM (ACU-DTM; Acuderm, Inc, Fort Lauderdale, Fla) cultures were performed in the office on part of the specimen. The remaining specimen was sent to the University of Texas Fungus Testing Laboratory at the University of Texas Health Science Center, San Antonio, for KOH evaluation and Sabouraud dextrose agar culture. The overall study design, disposition of specimens, and testing procedures are shown in the Figure. The Western Institutional Review Board, Olympia, Wash, approved the study protocol and all study materials.

Results of DTM culture were available in 2 weeks or less, and clinicians were informed of the culture results from the central laboratory 4 to 6 weeks after the sample was obtained. The initiation of antifungal therapy and the selection of a treatment were at the physician’s discretion. If the KOH test at the central laboratory was positive for fungal elements, but the culture result was negative, regardless of the DTM result, physicians were asked to obtain a second sample for a repeat culture. The study protocol excluded patients who were being treated from being retested. The primary analyses conducted in this study were (1) a paired comparison of in-office DTM culture and central laboratory fungal culture for each patient having results from both methods, and (2) a tabulation of the culture results and the infectious organisms that were identified.

MYCOLOGICAL EVALUATIONS

Physicians were supplied with DTM culture kits and a videotape to instruct them on how to obtain the nail bed sample and inoculate the DTM tube. The specimen was obtained after cleaning the surface of the nail plate with an alcohol swab and cut-
ting the nail plate with a sterilized curette or clipper to expose the nail bed. To increase the likelihood of detecting any dermatophytes that might be present, samples consisting of pieces of subungual debris from the proximal portion of the nail bed underneath the nail plate were obtained using a probe or curette. The specimen was divided, one piece was pressed lightly onto the culture medium in the DTM tube, and the cap was loosely applied to avoid sealing the tube off from the atmosphere. The specimen was incubated at room temperature for up to 2 weeks. The physicians checked the DTM culture daily for a change of color, which was interpreted as a positive result. False-positive results were avoided by examining the medium for the growth of white colonies typical of dermatophytes and by completing all readings by 14 days, after which time overgrowth by nondermatophyte fungi (other pathogens that can cause onychomycosis) might occur. Patients with a negative laboratory culture, regardless of the DTM result, were asked to submit a second specimen for retesting.

The Fungus Testing Laboratory performed its evaluations using well-established methods. Fungal culture was carried out using 2 media: 1 containing cycloheximide to inhibit nondermatophyte pathogens, and 1 cycloheximide-free medium, Sabouraud dextrose agar, to allow the growth of yeasts and nondermatophyte fungi (other pathogens that cause onychomycosis). Lack of growth of reproductive colonies within 4 to 6 weeks confirmed a negative fungal culture.

STATISTICAL ANALYSIS

The primary objective of this study was to determine the utility of DTM culturing to confirm a clinical diagnosis of onychomycosis. For evaluation of paired DTM and fungal culture results, positive DTM results were noted to agree with culture, regardless of the DTM result, were asked to submit a second specimen for retesting. Physicians were asked to obtain a second specimen for retesting if the KOH test was positive and the initial fungal culture was negative. False-positive results were avoided by examining the medium for the growth of white colonies typical of dermatophytes and by completing all readings by 14 days, after which time overgrowth by nondermatophyte fungi (other pathogens that can cause onychomycosis) might occur. Patients with a negative laboratory culture, regardless of the DTM result, were asked to submit a second specimen for retesting.

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RESULTS

One hundred forty-nine physicians enrolled at least 1 patient in the study. Eighty of the practitioners (54%) were podiatrists and the remaining 69 (46%) were primary care physicians. A total of 670 patients with signs and symptoms suggestive of onychomycosis were enrolled, 369 by podiatrists and 301 by primary care physicians. The comparison of in-office DTM culture and fungal culture is based on the 617 patients (92%) for whom complete data consisting of paired DTM and laboratory culture results were available by the cutoff for data collection. All 670 patients were included in the compilation of demographic and epidemiologic results.

Men and women were about equally represented. A majority of patients (73%) were white. Of note, 45% of the patients were 65 years or older and 17% were diabetic. A median of 4.8 toenails were affected, and 60% of patients had involvement of both feet. A similar average number of toes were affected on the right and left foot, 2.5 vs 2.4. Ten percent also had clinical evidence of fingernail involvement and 29% had symptoms of tinea pedis (Table 1). Fingernail involvement and the presence of tinea pedis were clinical observations only, and were not confirmed by DTM or fungal culture.

Central laboratory culture results were positive in 44% (n=297) of the patients. Three dermatophyte species (T rubrum, T mentagrophytes, and E floccosum) accounted for 93% of the positive cultures (Table 2). Nondermatophyte molds accounted for the remainder of
isolates, and no *Candida* infections were identified. No regional variations in the causative organisms of onychomycosis were found.

Dermatophyte test medium and laboratory cultures were in agreement (both positive [n=206] or both negative [n=214]) in 68% of the 617 patients for whom paired results were available at the time of data analysis, with a kappa coefficient of 0.37 (asymptotic SE, 0.04; 95% confidence interval, 0.299-0.441) (Table 3). Overall, the DTM cultures were positive in more cases than the laboratory cultures—51% (n=345) vs 44% (n=297).

Men had positive results by both culture methods more often than women (men, 64% for DTM and 59% for laboratory culture; women, 48% and 34%, respectively), but the differences were not significant. None of the other demographic variables were associated with the likelihood of a positive DTM or central laboratory culture result.

Laboratory cultures were negative in 342 patients. A second specimen was requested from these patients for retesting, and laboratory results were available for 105 patients at the time of data analysis. According to the study protocol, individuals receiving oral antifungal agents within the previous 90 days, or topical antifungal agents within the previous 30 days would not be included in the retest data. The retest culture was positive in 23 patients, 22% of the total who were retested. Of these, 10 were in patients who had a negative DTM culture, and 12 were in patients whose DTM culture was initially positive (Table 4). One retest culture grew a nondermatophyte mold. Retested patients were significantly more likely to be female than male (58% vs 42%; *P*=.05), but otherwise did not vary from the demographic profile of the entire population.

This large, prospective epidemiological study was based on a nationwide sample of patients in a community practice setting with signs and symptoms of onychomycosis. It is the largest study of this type to establish the concordance of DTM culture with central laboratory fungal culture on selective media for confirmation of a clinical diagnosis of onychomycosis. Central laboratory and DTM cultures were in agreement in 68% of the patients for whom paired results were available. Overall, the DTM cultures were positive in 51% of patients and central laboratory cultures in 44%. The kappa coefficient of 0.37, a measure of agreement between multiple tests, indicates a fair degree of agreement beyond that which would occur by chance. The investigation also confirmed dermatophytes as the primary pathogen in onychomycosis, accounting for 93% of infections, with nondermatophyte molds accounting for the rest. No cases of *Candida* onychomycosis were identified in the central laboratory cultures. In both the original cultures and the retested specimens, about 10% of the specimens that were DTM negative were positive by fungal culture, perhaps reflecting the longer time available for growth in cultures at the central laboratory.

Treatment for onychomycosis ideally should be based on a positive KOH test and a DTM or fungal culture showing recovery of a dermatophyte or other causative organism together with a clinical diagnosis including distally thickened toenails and, possibly, tinea pedis. This practice is based on the assumption that the mycological tests have a high positive predictive value in patients who have clinical signs of onychomycosis. In the present study, 403 patients (60%) with onychomycosis symptoms had positive results on either the laboratory or DTM culture—a recovery rate consistent with an earlier epidemiological study. Based on these results, fungal culture identified a maximum of 74% of true positives (297/403), while DTM culture identified 86% (345/403). The repeat cultures obtained in 105 patients suggest that negative results are not completely reliable; 22% (23/105) of patients with initially negative laboratory cultures were found to be positive for a dermatophyte on repeat cul-

### Table 2. Pathogens Identified by Central Laboratory Culture in 297 Patients With Onychomycosis Symptoms and Positive Cultures

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. (%) of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dermatophytes</td>
<td></td>
</tr>
<tr>
<td><em>Trichophyton rubrum</em></td>
<td>250 (84)</td>
</tr>
<tr>
<td><em>Trichophyton mentagrophytes</em></td>
<td>21 (7)</td>
</tr>
<tr>
<td><em>Epidermophyton floccosum</em></td>
<td>6 (2)</td>
</tr>
<tr>
<td>Nondermatophyte molds</td>
<td></td>
</tr>
<tr>
<td><em>Scopulariopsis brevicaulis</em></td>
<td>9 (3)</td>
</tr>
<tr>
<td><em>Paecilomyces lilacinus</em></td>
<td>3 (1)</td>
</tr>
<tr>
<td>All others</td>
<td>9 (3)</td>
</tr>
</tbody>
</table>

### Table 3. Comparison of In-Office DTM Culture and Central Laboratory Mycological Culture (N = 617) *

<table>
<thead>
<tr>
<th>DTM Culture</th>
<th>Central Laboratory Culture, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Positive</td>
<td>206 (33.4)</td>
</tr>
<tr>
<td>Negative</td>
<td>63 (10.2)</td>
</tr>
<tr>
<td>Total</td>
<td>269 (43.6)</td>
</tr>
</tbody>
</table>

*All percentages represent paired test results. A positive result (+) is defined as dermatophyte growth in fungal culture or a color change in DTM culture. A negative result (−) is defined as no growth or growth of a nondermatophyte in fungal culture, or lack of color change by 2 weeks in dermatophyte test medium (DTM) culture.

### Table 4. Patients With Completed DTM and Central Laboratory Culture Results Who Were Retested (n = 105)

<table>
<thead>
<tr>
<th>DTM Results</th>
<th>Central Laboratory Results</th>
<th>Retested Central Laboratory Results, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>Negative, 45 (43)</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Negative, 37 (35)</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>Negative, 82 (78)</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Positive, 10 (10)</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Positive, 12 (11)</td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>Positive, 23 (22)</td>
</tr>
</tbody>
</table>

*DTM indicates dermatophyte test medium.
†One of the 23 cultures was not a dermatophyte.

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from patients visiting a dermatologist for reasons other than nail disease, which originated with a study published in 1968,24 but appears unchanged to the present day.5,15,23 Estimates of the proportion of patients with clinical nail problems in whom a combination of the 2 standard techniques can confirm a diagnosis of onychomycosis range from about 60% to 65%.12,27 to as high as 80%.20 In this study, 60% of patients had dermatophyte-confirmed onychomycosis by either DTM or central laboratory culture.

Onychomycosis should be a concern of the primary care physician, and not only because its prevalence in the community makes it likely to be encountered in daily practice. Recent research has demonstrated the previously underestimated morbidity associated with onychomycosis. Extensive toenail infections can be painful, leading to difficulty standing or walking, limitations on wearing shoes, and consequent limitation of physical activity.27 Effects on overall function and quality of life have been documented, including reduced mobility and social activity in the elderly, reduced participation in leisure activities, and embarrassment or self-consciousness in social situations.27-29 In diabetic patients, onychomycosis was associated with a 3-fold risk in secondary bacterial infections, such as erysipelas, gangrene, and foot ulcers.30

Although the present study was not designed as an economic analysis, DTM is a relatively low-cost method to confirm a diagnosis of onychomycosis and is reimbursable. The cost of a DTM culture in this study was approximately $1 per test compared with $25 for each fungal culture performed at the central laboratory. Managed care providers often require a positive culture to reimburse treatment of onychomycosis. Dermatophyte test medium cultures satisfy that requirement and provide an economical way to assist in accurately confirming dermatophyte involvement to guide an expensive therapeutic course for physicians who would otherwise either treat empirically,
or avoid treating onychomycosis because of perceived difficulties in confirming a clinical diagnosis. If the cost, delay, and limited accuracy associated with current diagnostic methods are a barrier to treatment of onychomycosis, DTM cultures provide a way around that barrier.

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Corresponding author: Boni E. Elewski, MD, Department of Dermatology, University of Alabama at Birmingham, EFH 414, 1530 Third Ave South, Birmingham, AL 35294.

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