Onychomycosis

Clinical overview and diagnosis

Shari R. Lipner, MD, PhD, and Richard K. Scher, MD
New York, New York

Learning objectives
After completing this learning activity, participants should be able discuss the epidemiology of onychomycosis; recognize risk factors for onychomycosis; classify the organisms responsible for causing onychomycosis; recognize clinical features characterizing onychomycosis; discuss prognostic features that predict a poor response to treatment; define laboratory tests that are used to diagnose onychomycosis; and explain the advantages and disadvantages of each technique.

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Onychomycosis is a fungal nail infection caused by dermatophytes, nondermatophytes, and yeast, and is the most common nail disorder seen in clinical practice. It is an important problem because it may cause local pain, paresthesias, difficulties performing activities of daily life, and impair social interactions. In this continuing medical education series we review the epidemiology, risk factors, and clinical presentation of onychomycosis and demonstrate current and emerging diagnostic strategies. (J Am Acad Dermatol 2019;80:835-51.)

Key words: biofilm; confocal microscopy; dermatophyte; dermoscopy; direct microscopy; fungal culture; fungal nail infection; histopathology; nondermatophyte; onychomycosis; optical coherence tomography; polymerase chain reaction; tinea pedis; *Trichophyton rubrum*; yeast.

EPIDEMIOLOGY

Key points

- **The worldwide prevalence of onychomycosis is 5.5%**
- **Onychomycosis is less common in children and more common in older individuals**

Onychomycosis is the most common nail disease with a worldwide prevalence of 5.5% (based on a weighted average of 6 epidemiologic studies published in 2016).[1-6] The prevalence in the United States is estimated to be 2% to 14%.[7] A recent retrospective study of 36,634 children in San Diego between the ages of 12 and 18 years found a prevalence of onychomycosis of 1.1%.[6] Previous estimates worldwide were 0.44% to 2.6% in children.[8-10] Onychomycosis is common in older people and prevalence increases with age.[11-13] In 1 retrospective study of 8331 patients, the prevalence was 20.7% in adults >60 years of age.[12]

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Correspondence to: Shari R. Lipner, MD, PhD, Department of Dermatology, Weill Cornell Medicine, 1305 York Ave, New York, NY 10021. E-mail: shl9032@med.cornell.edu.
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Abbreviations used:

ELISA: enzyme-linked immunosorbent assay
EO: endonyx onychomycosis
GMS: Grocott methanamine silver
KOH: potassium hydroxide
MPO: mixed pattern onychomycosis
NDM: nondermatophyte mold
PAS: periodic acid–Schiff
PCR: polymerase chain reaction
PSO: proximal subungual onychomycosis
SO: superficial onychomycosis
TDO: total dystrophic onychomycosis

RISK FACTORS

Key points

- **Trauma, older age, diabetes, and immunosuppression predispose to onychomycosis**
- **Tinea pedis, psoriasis, and family history of onychomycosis are other risk factors**

Well-recognized risk factors for onychomycosis are trauma,[14] advancing age,[11,15] and a history of tinea pedis.[16,17] Comorbidities, such as diabetes,[18,19] obesity,[20-22] immunosuppression,[23-25] and malignancies,[19] are also associated with increased risk. The presence of onychomycosis is common among related family members and may be autosomal dominantly inherited. Human leukocyte antigen–DR8 is more common in people with onychomycosis than normal controls.[26] Dermatophyte foot and toenail infections are common among members of the same household, and the risk of transmission to other family members when 1 member is affected is 44% to 47%.[27,28] Onychomycosis is also more prevalent in psoriatic patients than healthy controls,[29] and it is estimated that psoriatics have a >50% chance of developing onychomycosis compared with nonpsoriatics.[30] Table I displays risk factors associated with onychomycosis.

PATHOGENIC ORGANISMS

Key points

- **Dermatophytes, particularly *Trichophyton rubrum*, are responsible for most fungal nail infections**
- **About 30% to 40% of onychomycosis cases are caused by nondermatophyte molds and yeasts**
- **Biofilms have recently been recognized to play an important role in the pathogenesis of onychomycosis**

Onychomycosis is caused by dermatophytes, nondermatophyte molds (NDMs), and yeasts. More than 60% to 70% of these infections are caused by dermatophytes, predominantly *T rubrum* (>50%) and *Trichophyton mentagrophytes* (about 20%), with remaining infections caused by *Epidermophyton floccosum*, *Microsporum* spp., *Trichophyton violaceum*, *Trichophyton verrucosum*, *Trichophyton krajdenii*, and *Arthroderma* spp.[2,33,39] NDMs are responsible for approximately 20% of fungal nail infections and the most common organisms are *Scopulariopsis brevicaulis*, *Aspergillus* spp., *Acremonium*, *Fusarium* spp., *Alternaria alternate*, and *Neoscytalidium*.[40] Yeasts, including *Candida* spp., account for 10% to 20% of cases of onychomycosis.[2,33] Onychomycosis caused by ≥2 fungal organisms are being increasingly identified with molecular biology,[41] and bacterial–fungal infections are relatively common.[42,43]

While fungi were previously believed to be planktonic (in suspension, free-floating, and acting independently),[44] recent evidence supports the formation of biofilms.[45] Biofilms are sessile microbial communities that attach to biological surfaces, such as the nail plate, via an extracellular matrix that encases them.[45] In doing so, they may develop resistance to antifungal drugs,[46] exhibit increased virulence,[47] and evade the immune system.[48] Dermatophytes, including *T rubrum* and *T mentagrophytes*,[49] NDMs including *Aspergillus fumigatus* and *Fusarium* spp.,[50,51] and yeast such as *Candida albicans*[45] all form biofilms in vitro. In addition, *T rubrum* and *T mentagrophytes* begin forming biofilms at 3 hours that are fully formed by 72 hours (Fig 1).[49] It is believed that biofilms play an important role in treatment resistance.[52]

CLINICAL PRESENTATION

Key points

- **Onychomycosis has physical and social consequences**
- **The most common presentation of onychomycosis is yellowing, onycholysis, and subungual hyperkeratosis**
- **The most common dermoscopic pattern of onychomycosis is a jagged proximal border with spikes in the onycholytic area**

History

Patients with onychomycosis often complain of nail discoloration, nail separation, brittleness, or thickening that often worsens with time.[53] A history of tinea pedis or hyperhidrosis of the feet is common.[16,17,32] Nails affected by onychomycosis may cause local pain, difficulty in fitting shoes and with employment, social

Table I. Risk factors associated with onychomycosis

Dermatologic conditions	Comorbidities	Exogenous factors	Other
Tinea pedis[16,17]	Diabetes[18,19]	Trauma[14]	Advancing age[11,15]
Psoriasis[29,30]	Immunosuppresion,[23-25] including HIV, chemotherapy, transplant, and dialysis	Poor nail grooming[31]	Genetics[26]
Hyperhidrosis[32]	Venous insufficiency[12,33]	Sports and fitness activities[12]	Hallux valgus[12]
	Malignancies[19]	Occupation[31]	Asymmetric gait nail unit syndrome[34]
	Peripheral artery disease[35,36]	Smoking[36]	Contact with other household members[28]
	Obesity[20-22]	Occlusive shoes[37]	
	Inflammatory bowel disease[38]		

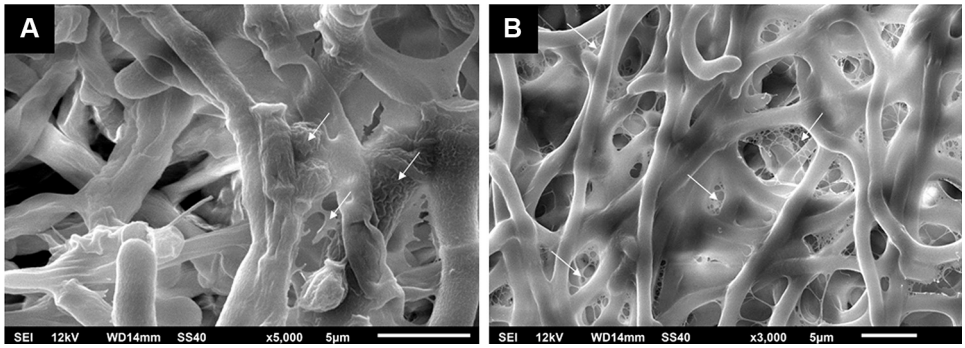

Fig 1. Scanning electron microscopy of mature fungal biofilms formed in 24-well plates. White arrows denote extracellular matrix covering and connecting the hyphae. **A**, *Trichophyton rubrum* ATCC 28189. **B**, *Trichophyton mentagrophytes* ATCC 11481. Photographs courtesy of Dr Caroline Costa Orlandi.

embarrassment, and have a negative impact on quality of life.[31] Nail conditions may not be mentioned by the patient as the chief complaint because of the misperception of bringing up something trivial. Therefore, it is imperative that the examining physician specifically asks about any nail problems. A medication list (oral, topical, and over the counter medications) should be obtained, including previous antifungal medications and start and stop dates because it may impact diagnostic testing and therapy.

Physical examination

In evaluating a patient with nail disease, all 20 nails should be examined as well as the hands and feet. Patients are instructed to remove all nail polish before the examination. Onychomycosis most commonly involves the toenails, with the great toenail most frequently affected.[53] It is unusual to have involvement of ≥1 fingernail(s) without concomitant toenail involvement unless there is a history of trauma or immunosuppression. Typical physical examination findings include hyperkeratosis of the nail bed, which often causes varying degrees of nail plate onycholysis (Fig 2, *A*). A white **[F2-4/C]** or yellow discoloration of the nail plate is common as well as subungual debris (Fig 2, *B* and *C*). In long-standing or severe cases there may be extensive onychodystrophy with nail plate thickening, crumbling, ridging, onychocryptosis, and partial or complete nail loss (Fig 2, *D*). In many cases, there is scale in the web spaces or plantar surfaces of the feet (Fig 2, *E*). Trauma is a risk factor for onychomycosis, and violaceous/brown/black nail plate discoloration may also be present. A dermatophytoma, or fungal abscesses, is a white/yellow or orange/brown longitudinal streak in the nail plate and is quite specific for onychomycosis (Fig 3).[54] On histology, there are large numbers of clustered, thick-walled fungal hy- **[F3-4/C]** phae.[55] *Scytalidium dimidiatum*, *Aspergillus niger*, and *A alternate* produce diffuse brown to black

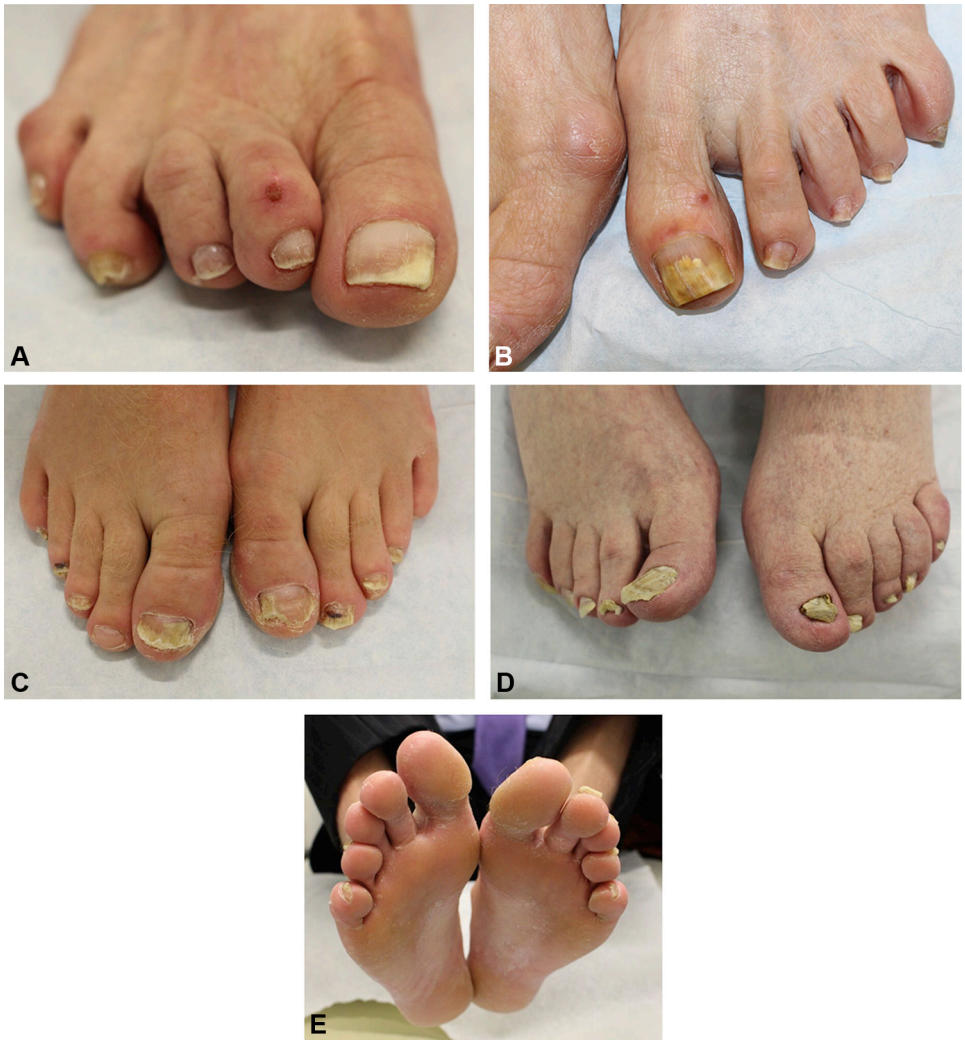

Fig 2. Physical examination findings in onychomycosis. **A**, Right great toenail with subungual hyperkeratosis and nail plate onycholysis. **B**, Left great toenail with yellow discoloration and onycholysis. **C**, Multiple toenails with subungual hyperkeratosis and onycholysis. **D**, Toenails with severe onychodystrophy and ridging. **E**, Scale on the plantar feet and web spaces.

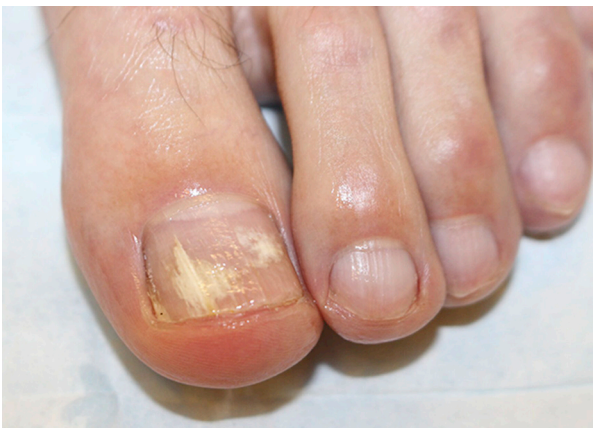

Fig 3. Dermatophytoma. White-yellow longitudinal streak in the nail plate.

pigmentation of the nail plate.[56] The dermatophyte *T rubrum var. nigricans* instead causes longitudinal melanonychia in which the band is usually wider distally and narrows proximally (Fig 4).[57] **[F4-4/C]** Longitudinal melanonychia, because of benign melanocytic activation, may be caused by *Candida* species, particularly in patients with higher Fitzpatrick skin types.[58] However, a biopsy specimen should be obtained from any concerning melanonychia to rule out subungual melanoma. Onychomycosis may coexist with other nail malignancies, and therefore significant nail dystrophy or failure to improve with antifungal therapy, despite laboratory evidence of a fungal infection, should prompt obtaining a biopsy specimen from a nail.[59,60]

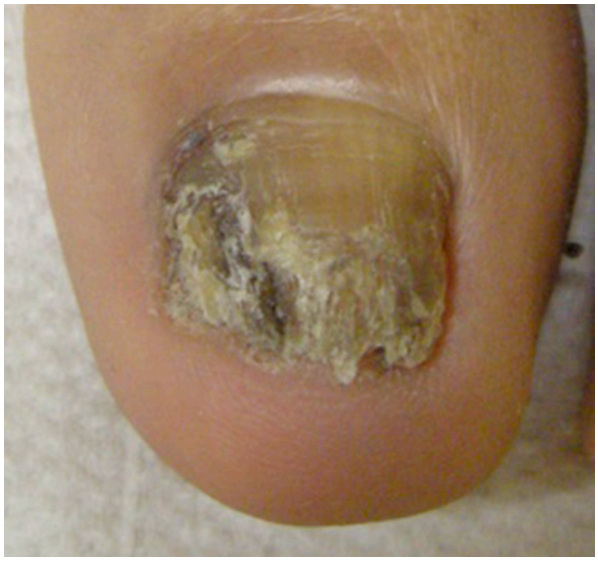

Fig 4. Fungal melanonychia. Onychodystrophy and longitudinal melanonychia caused by *Trichophyton rubrum* var *nigricans*. The pigmented band is wider distally and tapers proximally. Photograph courtesy of Dr Maria Bianca Piraccini.

Dermoscopy

Dermoscopy is a helpful noninvasive diagnostic tool that can be used to differentiate onychomycosis from both traumatic onycholysis and melanonychia.[61,62] The most common pattern consistent with a diagnosis of onychomycosis is a fringed proximal **[F5-4/C]** border in the area of onycholysis (Fig 5, *A*).[62] Another typical pattern is longitudinal striae, characterized by vertical streaks of different colors in the onycholytic area resembling an aurora borealis (Fig 5, *B*).[62] The discoloration may be distributed around striae or patches (Fig 5, *C*). In addition, there may be a ruin-like appearance, defined as subungual hyperkeratosis and debris (Fig 5, *D*).[63,64] In cases of fungal melanonychia, dermoscopy may show linear bands that are rounded proximally and tapered distally.[56] Other important dermoscopic features present in fungal melanonychia are yellow or **[F6-4/C]** multicolored patterns (Fig 6, *A* and *B*), non-longitudinal homogenous or reverse triangular patterns, subungual keratosis, white or yellow streaks, and scales on the nail plate, while longitudinal patterns and pseudo-Hutchinson sign are rarely seen.[65]

CLINICAL CLASSIFICATION

Key points

- **Distal lateral subungual onychomycosis is the most common clinical pattern**
- **Proximal subungual onychomycosis is associated with immunosuppression**

Onychomycosis is divided into subtypes based on the pattern of invasion. Distal lateral subungual onychomycosis is the most frequent type and is characterized by distal onycholysis with subungual hyperkeratosis, nail plate thickening, and yellow to brown discoloration (Fig 7, *A*). In fingernails, **[F7-4/C]** minimal hyperkeratosis with prominent onycholysis may occur. In superficial onychomycosis (SO), the infecting organisms invade the upper surface of the nail plate and are most often seen as superficial white patches that are easily scraped off (Fig 7, *B*).[66] Less common presentations are black patches, transverse striate, and invasive patterns.[67] SO is seen more often in immunosuppressed patients, particularly in those with HIV.[68] Proximal subungual onychomycosis (PSO) develops when the fungus invades from the undersurface of the proximal nail fold and then progresses distally. On physical examination, there are diffuse patches or a transverse striate pattern (Fig 7, *C*). When PSO progresses quickly, it may be associated with immunosuppression.[69] Endonyx onychomycosis (EO) is caused by fungal invasion of the nail plate without infection of the nail bed. While the other onychomycosis subtypes are most commonly caused by *T rubrum*, EO is frequently caused by *Trichophyton soudanense* and *T violaceum*. Characteristic findings are lamellar nail splitting and milky patches of the nail plate (Fig 7, *D*).[69] In mixed pattern onychomycosis (MPO) there is >1 type of nail plate infection within the same nail; most commonly PSO or distal lateral subungual onychomycosis combined with SO.[69] Total dystrophic onychomycosis (TDO) is the end stage of onychomycosis and presents with a severely dystrophic and crumbled nail plate.[69] Secondary onychomycosis is defined as fungal invasion following another nonfungal nail condition, such as psoriasis or previous trauma (Fig 7, *E*). Clinically, it resembles both the fungal and nonfungal nail conditions.[69]

DIAGNOSTIC TESTING

Key points

- **Confirmatory testing should be performed before initiating treatment for onychomycosis**
- **Direct microscopy, fungal culture, and histopathology may be used for confirmation**
- **Polymerase chain reaction may be used to rapidly identify the infecting organism**

While history, physical examination, and dermoscopy are helpful in diagnosing onychomycosis, mycologic laboratory confirmation is necessary for definitive diagnosis. Empiric treatment of

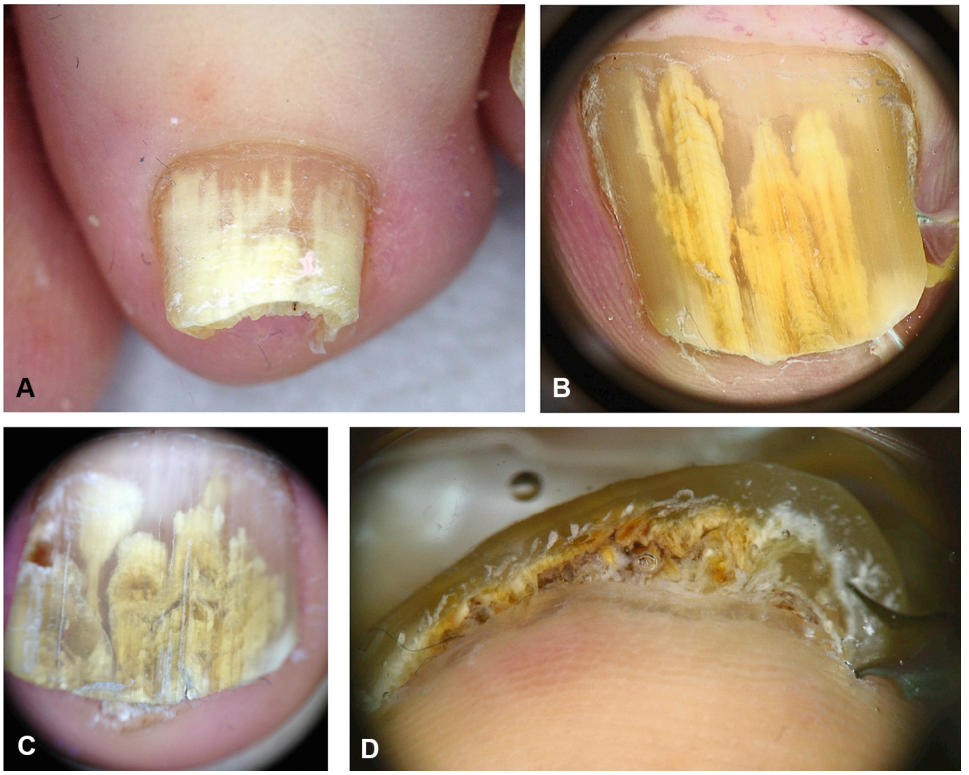

Fig 5. Dermoscopy of onychomycosis. **A**, Fringed proximal margin of the onycholysis. **B**, Blurred yellow-orange-brown nail discoloration in longitudinal striae (the fading mimics Aurora Borealis). **C**, Distribution of the discoloration in longitudinal striae or round areas. **D**, Ruin-like appearance of the subungual scales that are white-yellow-orange in color. Photographs courtesy of Dr Maria Bianca Piraccini.

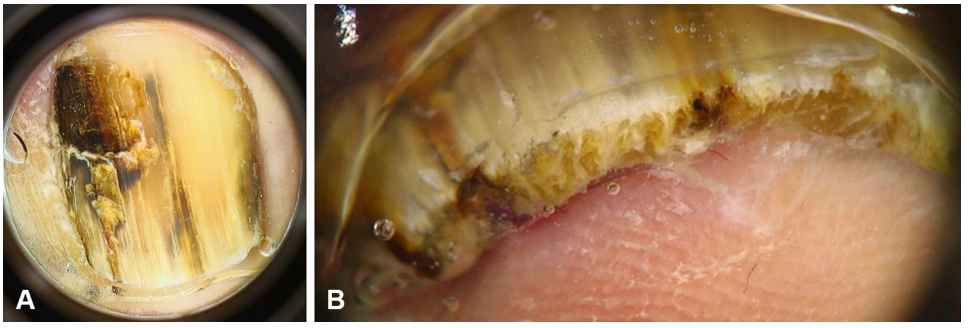

Fig 6. Dermoscopy of fungal melanonychia. **A**, Different colors of nail discoloration, including black, yellow, white, and orange. **B**, Black, white, yellow, and orange discoloration of subungual scales. Photographs courtesy of Dr Maria Bianca Piraccini.

onychomycosis is still performed by many general practitioners, podiatrists, and some dermatologists.[70,71] Some argue that treating nail fungal infections empirically with terbinafine is cost effective and that bypassing testing allows patients to be treated promptly.[72] However, another recent study found that confirmatory testing was indeed cost effective after all.[73] Mycologic testing is critical to avoid treatment failures, incorrect diagnoses, unnecessary side effects, and potential drug–drug interactions.[74-76] Table II shows other conditions that may clinically mimic onychomycosis.

The ideal mycologic test would identify the infecting organism, determine viability, be easy to perform with rapid results and a low cost, and be highly sensitive and specific. Currently available techniques are microscopy, fungal culture, histopathology, molecular biology, and combinations of these techniques.[77] To obtain a result that is clinically meaningful, nail sampling should be performed after

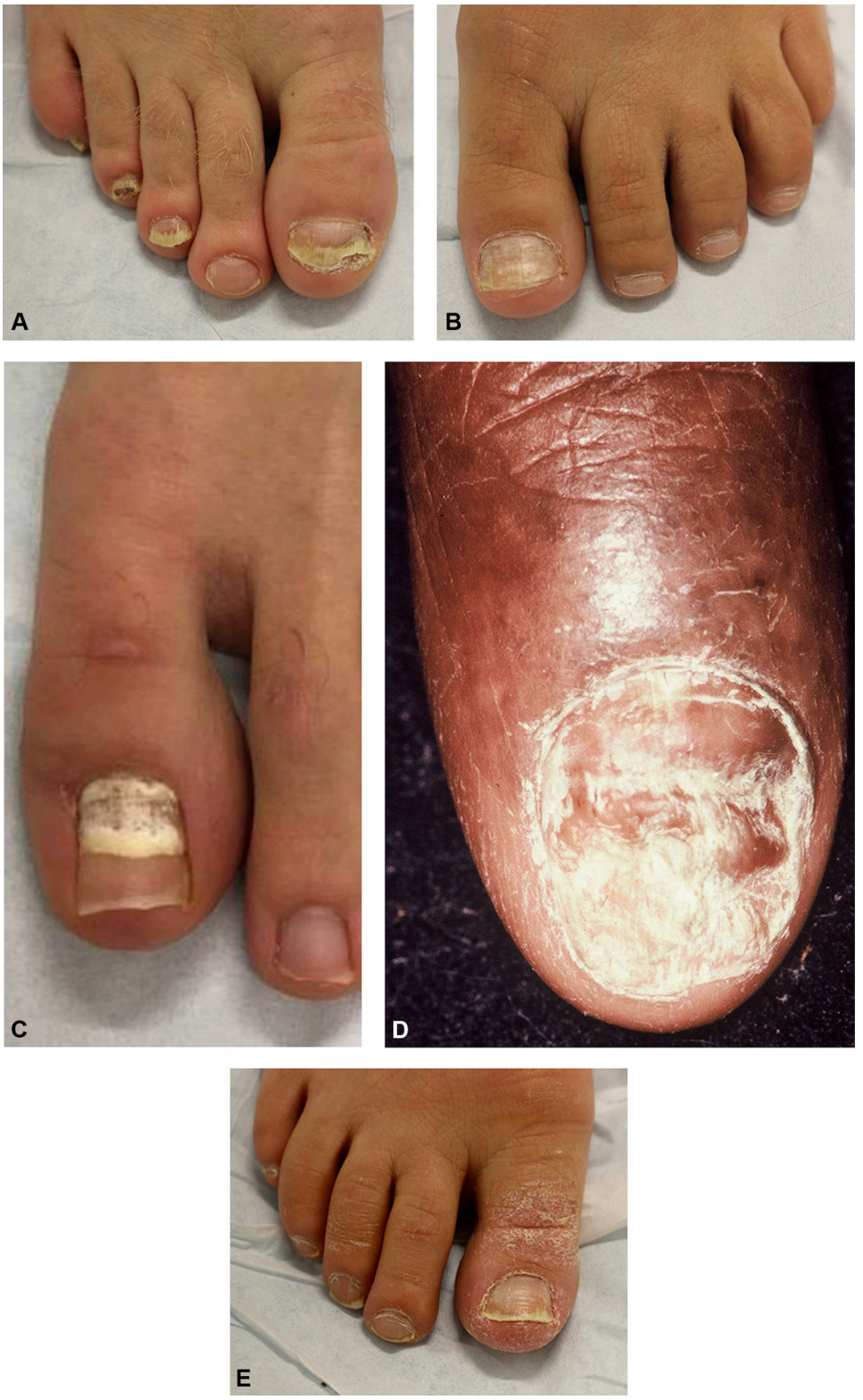

Fig 7. Clinical classification of onychomycosis. **A**, Distal lateral subungual onychomycosis. Right great toenail and third to fifth toenails with yellowing, distal onycholysis, and subungual hyperkeratosis. **B**, Superficial onychomycosis. Left great toenail with superficial white patches of the nail plate. **C**, Proximal subungual onychomycosis. Left great toenail with diffuse white patches involving the proximal nail plate. **D**, Endonyx onychomycosis. Lamellar nail splitting and milky patches of the nail plate. **E**, Secondary onychomycosis. Well-defined erythematous plaque with on the right great toe and right great toenail nail plate with pitting, onycholysis, and a salmon patch. Histopathology showed fungal hyphae. Endonyx onychomycosis photograph courtesy of Dr Robert Baran.

Table II. Differential diagnosis of onychomycosis[53,74,75]

Benign conditions	Malignant conditions
Nail psoriasis	Subungual squamous cell carcinoma
Nail lichen planus	Subungual melanoma
Subungual and periungual verruca	
Paronychia	
Subungual exostosis	
Onychomatricoma	
Yellow nail syndrome	
Idiopathic or traumatic onycholysis	

a 3- to 6-month washout period of previous antifungals, including oral, topical, and over the counter medications. Otherwise, the drug may be retained in the subungual debris and be transferred to the culture media, thereby inhibiting fungal growth.[78]

The clinical presentation determines the site of sample collection. For example, for distal lateral subungual onychomycosis, the sample is obtained from the most proximal area of involvement after removal of the distal area of onycholysis (Fig 8, *A*). For PSO, the nail plate is gently debrided to sample the underlying nail debris. For SO, scrapings are taken from the affected nail plate.[79] A double action nail clipper is a useful tool for obtaining samples for fungal examinations, especially when the nail is >1 mm in thickness, which is common in fungal nail infections.[80] This clipper generates a large force at the point of clipping, thereby requiring minimal strength by the physician and minimizing patient discomfort (Fig 8, *B*).[81]

[F8-4/C]

Potassium hydroxide and microscopy

For microscopic examination, the onycholytic nail is clipped back as far as possible with a sterile nail clipper and the subungual debris is scraped onto a glass slide with a no. 1 curette. The addition of potassium hydroxide (KOH) serves to dissolve larger keratinocyte material, making it more flat and diminishing reflection of cell borders, with light microscopy used to determine the presence or absence of fungal elements (Fig 9, *A*).[53] An advantage of this technique is that it can be performed in the office setting within minutes. However, this technique lacks sensitivity, cannot determine viability, and is expertise-dependent.[82,83] Sensitivity is even worse for the detection of NDMs.[83] In addition, fat droplets, air bubbles, and cotton fibers may mimic fungal hyphae when KOH is used (Fig 9, *B*).[84] Performing successive KOH examinations up to 3 times may increase sensitivity up to twofold.[85] Fumes from KOH on slides may damage microscope optics, and therefore it is advisable to buy a less expensive microscope for this purpose. An alternative stain is Parker blue black ink, which is visualized with a light microscope, and calcofluor white, which is used in conjunction with a fluorescent microscope. Calcofluor white binds to dermatophyte, NDM, and *Candida* beta 1-3 and beta 1-4 polysaccharides and fluoresces with ultraviolet light radiation, thus augmenting the visibility of fungal hyphae (Fig 10).[86]

[F9-4/C]

[F10-4/C].

Fungal culture

Fungal culture is currently the only technique that can definitively identify the causative organism and its viability and is thus still considered the criterion standard in diagnosing onychomycosis.[87] Thorough cleaning of the nail with both 70% isopropyl alcohol and soap and water is essential because colonizing organisms can confound culture results and inhibit growth of the relevant pathogen.[87] The nail plate is then clipped back, and the subungual debris is scraped onto a clean 2- × 2-in gauze or small piece of cardboard and sent to the laboratory.[53] To avoid false negative results, an adequate amount of scrapings must be sent for evaluation.[88] Subungual debris is preferred over nail clippings because the latter may harbor bacterial or nonpathogenic mold contaminants that can overgrow the true pathogen on culture media.[87] Most laboratories use 2 types of media: an agar containing cycloheximide to inhibit nondermatophytes and encourage growth of dermatophytes, and an agar without cycloheximide to culture nondermatophytes (Fig 11, *A*).[89,90] Chloramphenicol and gentamicin are typically added to the agar to reduce bacterial contaminants (Fig 11, *B*).[89] The culture is grown at 25°C to 30°C for up to a month (Fig 12). Limitations of this technique are the time it takes to get results (several weeks) and a high false negative rate.[77]

[F11-4/C].

[F12-4/C].

Histopathology

Histopathology on a nail clipping is an easy, sensitive, and relatively rapid method to diagnose onychomycosis. Compared with KOH and fungal culture, it is the most sensitive technique.[84,86] Hematoxylin–eosin staining can be used to visualize fungal elements (Fig 13, *A*). However, because the hyphae are enhanced with periodic acid–Schiff (PAS) or Grocott methanamine silver stains, with similar sensitivities, they are preferred over hematoxylin–eosin staining.[53,91,92] Periodic acid oxidizes hydroxyl groups of polysaccharides in the

[F13-4/C].

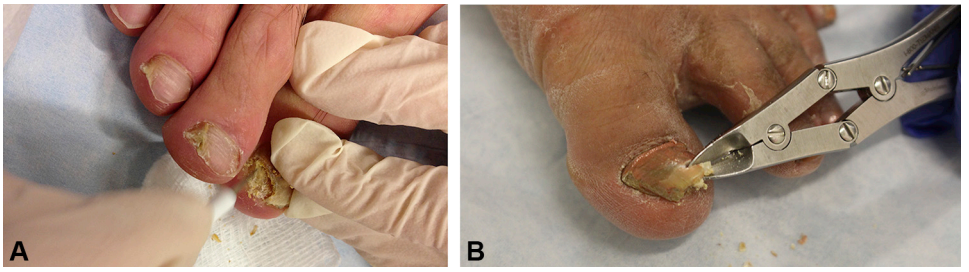

Fig 8. Sample collection. **A**, For distal lateral subungual onychomycosis, a no. 1 curette is used to gently scrape the subungual debris after removal of the distal area of onycholysis. **B**, A double action nail clipper is used to sample a thickened nail plate.

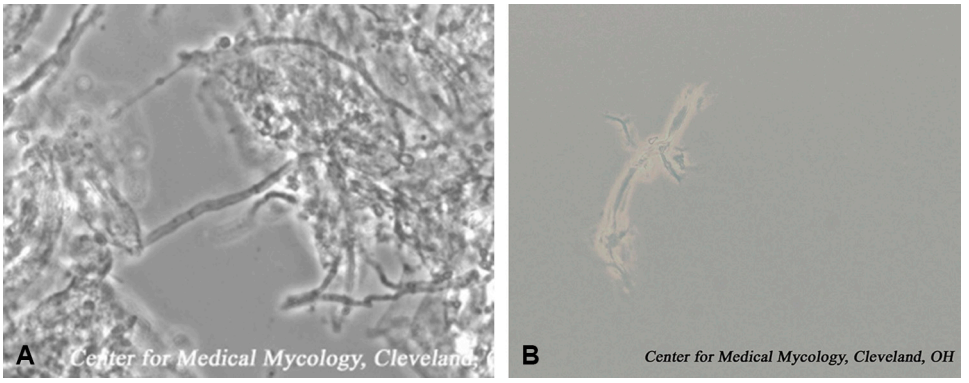

Fig 9. Potassium hydroxide (KOH) with direct microscopy. **A**, Septate hyphae from pure culture in KOH, visualized under white light. **B**, Cotton fiber artifact seen on KOH direct smear. It is differentiated from dermatophyte hyphae by the lack of septations and parallel sides. (Original magnification: *A* and *B*, ×40.) Photographs courtesy of Center for Medical Mycology.

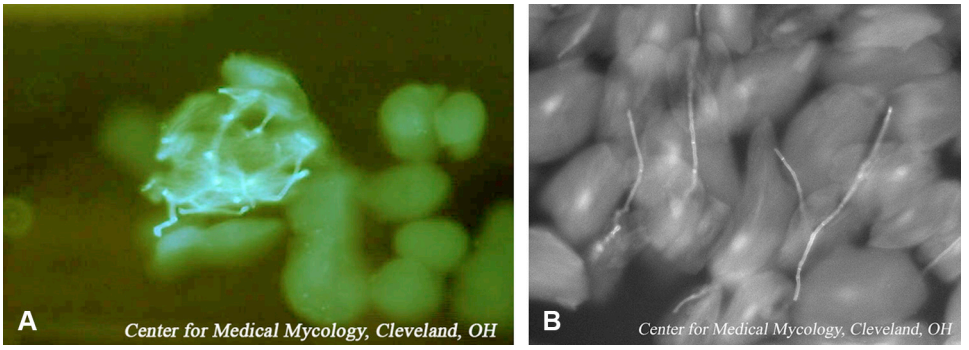

Fig 10. Dermatophyte hyphae in subungual debris, stained with calcofluor white and visualized with fluorescent microscopy. **A**, Color photograph. **B**, Black and white photograph highlighting septations. (Original magnification: *A* and *B*, ×40.) Photographs courtesy of Center for Medical Mycology.

fungal cell wall into aldehyde, which then reacts with the Schiff reagent. As a result, the background remains green, and the fungal elements stain red (Fig 13, *B*). For Grocott methanamine silver staining, chromic acid oxidizes polysaccharides in the fungal cell wall to aldehydes, and methenamine silver nitrate is then reduced to metallic silver. The background is pale green while the fungal elements are dark brown (Fig 13, *C*). Alternative stains are Fontana–Masson, hematoxylin–eosin, immunofluorescence, and Mayer mucicarmine.[93] Histopathology can be easily used to identify hyphae, pseudohyphae, spores, and yeast. Disadvantages of this technique are that the identity and viability of the pathogen cannot be identified.[86] Histopathology, although not unique to nail specimens, is associated with risks to laboratory personnel because of exposure to known carcinogens and the skin irritants phenol and formalin

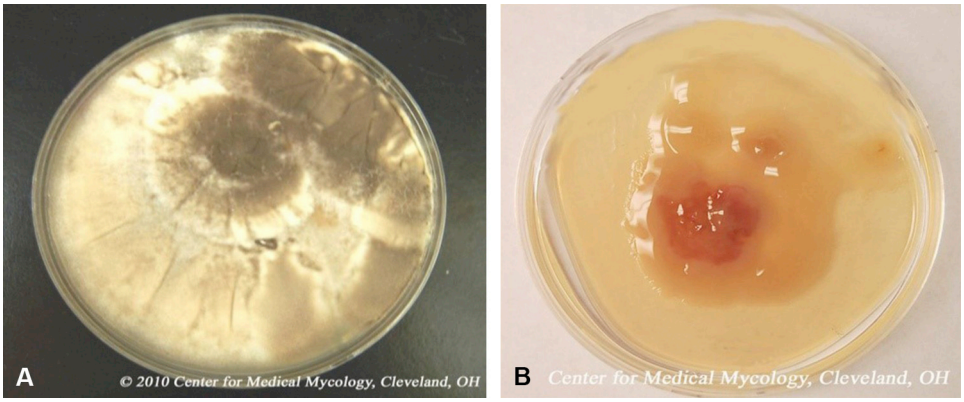

Fig 11. Cultures overgrown by saprophytic mold and bacteria. **A**, Clinical sample plated on potato dextrose agar plate, overgrown by saprophytic mold after 1 week. **B**, Clinical sample plated on mycosel agar, overgrown by bacteria after 4 to 5 days. Photographs courtesy of Center for Medical Mycology.

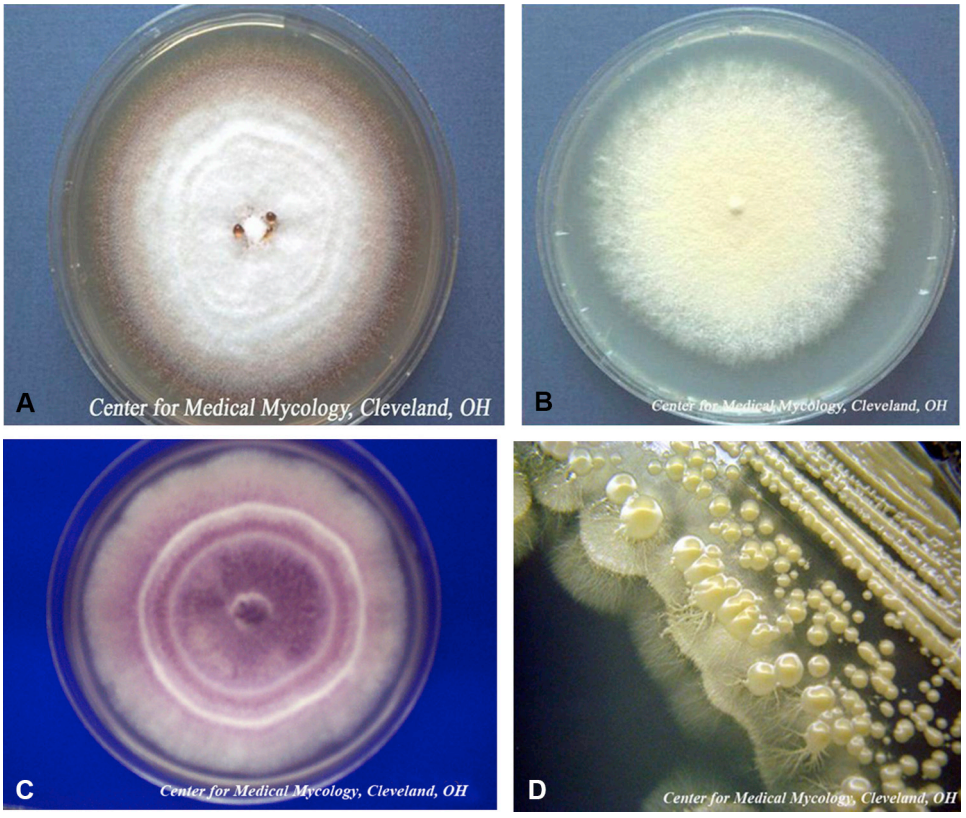

Fig 12. Fungal colonies on potato dextrose agar. **A**, *Trichophyton rubrum* colony with formation of red pigment. **B**, *Trichophyton mentagrophytes* colony with powdery surface and beige pigment. **C**, Fusarium colony. **D**, *Candida albicans* colony showing mycelial growth after 2 weeks. Photographs courtesy of Center for Medical Mycology.

and must be handled with adequate protection and a chemical hood.[87]

Polymerase chain reaction

Polymerase chain reaction (PCR) is the newest addition to our armamentarium of laboratory tests to diagnose onychomycosis. It is a DNA-based strategy that can be used to identify dermatophytes, nondermatophytes, and *Candida* spp. using specific primers.[94] One commonly used target is the gene fragment of the fungal small ribosomal subunit 18S rRNA, which is present in fungi but

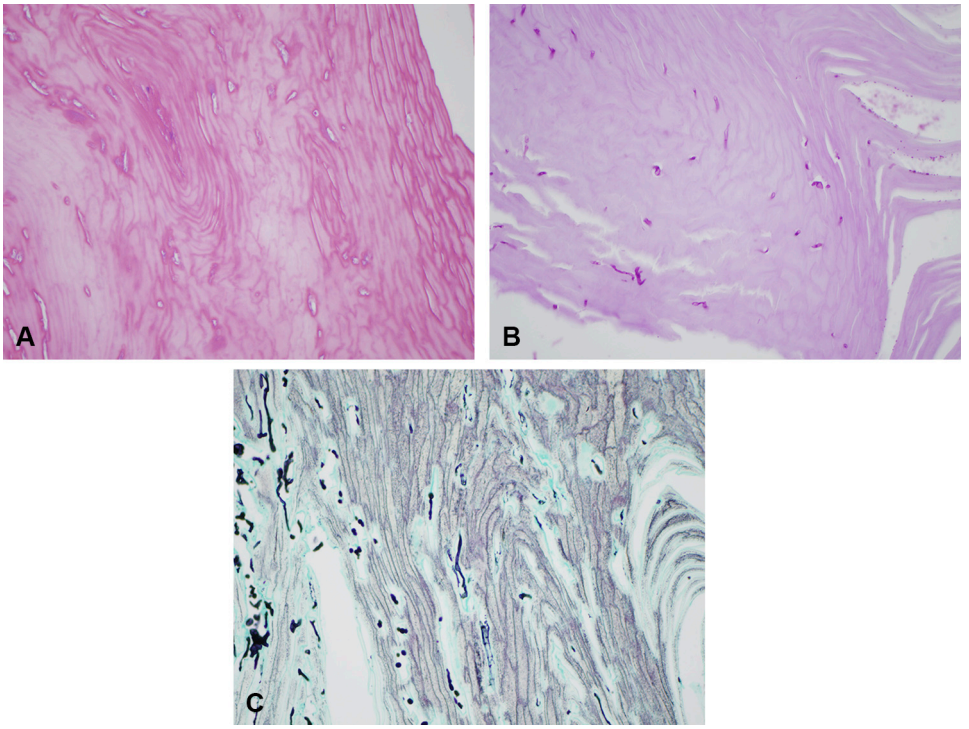

Fig 13. Histopathology of onychomycosis. **A**, *Trichophyton rubrum* onychomycosis with hematoxylin–eosin staining. Nail plate and subungual horn with hyperkeratosis. There are faint hyphae coursing through the keratin, even at high power. **B**, *T rubrum* onychomycosis with periodic acid–Schiff staining and visualization of the hyphae infiltrating the subungual horn. **C**, *T rubrum* onychomycosis with Grocott methanamine silver staining and enhancement of the fungal hyphae. The identity of the infecting organism was later confirmed by culture. (Original magnification: *A-C*, ×60.) Photographs courtesy of Drs Cynthia Magro and Allen Miraflor.

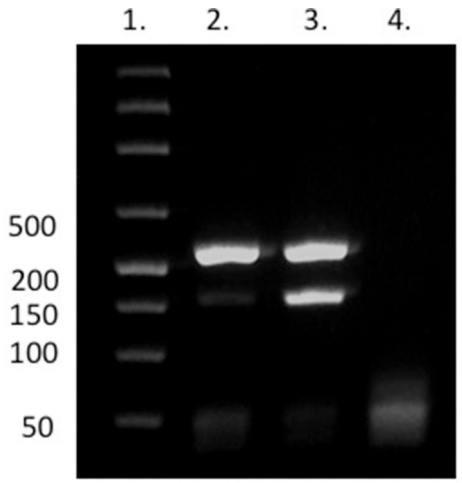

Fig 14. Gel electrophoresis of DNA fragments generated by polymerase chain reaction. Lane 1, DNA ladder; lane 2, *Trichophyton rubrum* amplicon (213 base pairs); lane 3, *T rubrum* amplicon (213 base pairs) and *Candida albicans* amplicon (156 base pairs); lane 4, negative control. Photograph courtesy of Bako Pathology Services (Alpharetta, GA).

not humans.[95] Using ≥2 restriction enzymes to digest amplicons allows for differentiation between dermatophytes, yeasts, and molds (Fig 14).[96-98] A variation on this technique is the combination of PCR with enzyme-linked immunosorbent assay, in which nail genomic DNA is first amplified with PCR and then detected using biotin-labeled probes. Microarrays, restriction fragment length polymorphism, and PCR terminal restriction fragment length polymorphism can be used to detect onychomycosis caused by multiple organisms.[41,99] Results are typically available within a few to 48 hours depending on laboratory volume. This test is becoming more widely available and covered by many insurance plans. Real-time PCR is a similar technique, but can quantitate relative amounts of transcripts, which translates into information about probable viability of the organism.[99,100] Commercial PCR kits are also becoming more widely available, cost effective, and have a high sensitivity and specificity in diagnosing onychomycosis.[101-103]

Table III. Comparison of techniques used for diagnosis of onychomycosis[53,87]

Technique	Nail plate penetrance	Fungal viability	Identification of pathogen	Sensitivity (%)	Specificity (%)	Cost per examination (approximate)	Dependent on expertise of examining physician*	Commonly used
KOH	No	No	No	67-93	38-78	$6-11[72,73]	Yes	Yes
Fungal culture	No	Yes	Yes	31-59	83-100	$14-48[72‡]	No	Yes
Histopathology with PAS	Yes	No	No	92	72	$29-365[72,73‡§]	No	Yes
PCR[†]	No	No	Yes	95	100	$30/probe[‡]	No	Increasing

KOH, Potassium hydroxide; *PAS*, periodic acid—Schiff; *PCR*, polymerase chain reaction.
*Note that results of histopathology are dependent on the expertise of the dermatopathologist and the results of fungal culture and polymerase chain reaction studies are dependent on the microbiology laboratory.
[†]It is typical that 3 probes (dermatophyte, saprophyte, and Candida) are run initially. If negative, no further testing is performed. If the test is positive, further testing is performed for the specific species. For example, if the sample is initially positive for dermatophytes, further testing is performed for *Trichophyton rubrum*, *Trichophyton mentagrophytes*, Microsporum, and Epidermophyton.
[‡]Bako Pathology, personal communication, August 21, 2017.
[§]Weill Cornell Pathology, personal communication, March 1, 2018.

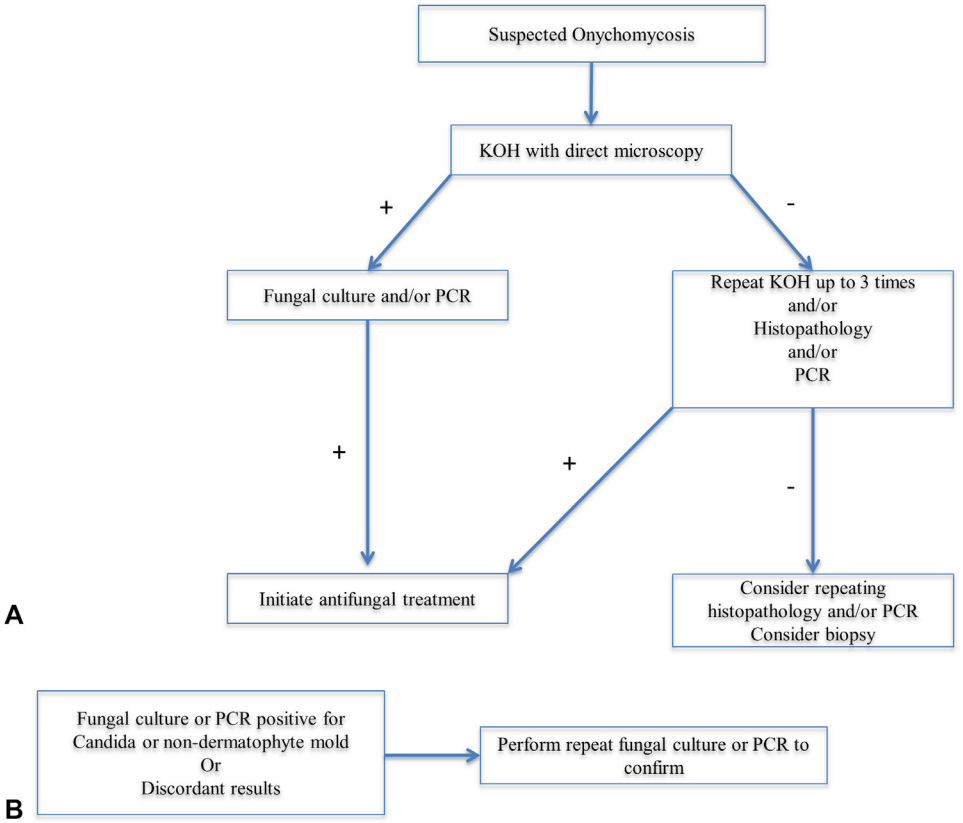

Fig 15. Algorithms for diagnosis of onychomycosis. **A**, Suggested algorithm for the diagnosis of suspected onychomycosis. **B**, Suggested algorithm for fungal culture or polymerase chain reaction study that is positive for Candida, nondermatophyte mold, or discordant results.

Comparison of techniques

Currently available diagnostic techniques include KOH with microscopy, fungal culture, histopathology, and PCR. One or more techniques can be used to diagnose onychomycosis, and the method(s) chosen is dependent on patient characteristics, time to initiate therapy, cost, sensitivity and specificity of the technique, and expertise of the clinician. The KOH examination is a rapid and cost-effective method for diagnosing onychomycosis, but it is highly expertise-dependent. Nail psoriasis may clinically resemble or coexist with onychomycosis.

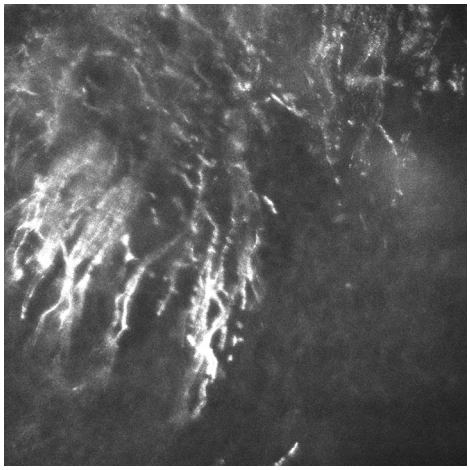

Fig 16. Reflectance confocal microscopy. Confocal image of septate hyphae in toenail onychomycosis. (Vivascope 3000; original magnification, ×700.) Photograph courtesy of Dr Philippe Bahadoran.

If this differential diagnosis is being considered, histopathology may be the best diagnostic option because it can be used to both visualize the presence or absence of hyphae and rule in or out nail psoriasis. If the patient is immunosuppressed and an NDM is a possibility, PCR can be used to quickly identify the pathogen and initiate treatment while a culture is simultaneously performed to confirm the identity and viability of the pathogenic organism. PCR is 3 to 4 times less likely to yield a false-negative result compared with fungal culture,[104] and highlights the need to obtain serial samples, particularly in cases of NDM infections. Table III shows a comparison of diagnostic techniques.

Combinations

A combination of techniques can be used to diagnose onychomycosis to give more information on the presence or absence of hyphae and the viability and identity of the organism. For example, KOH is commonly used with fungal culture in the office setting. If a given KOH is positive but the fungal culture is negative, the culture may be repeated because of the high false negative rate for cultures.[77] Mycologic cure is defined as a negative KOH and negative culture. The US Food and Drug Administration requires this endpoint in onychomycosis studies, and therefore KOH and culture are often reported together in clinical trials.[78,105] Another accepted but rarely performed combination is KOH and PAS staining (KONCPA). KOH 20% solution is added to the nail specimen, incubated, and centrifuged, and the pellet is analyzed for fungal elements after staining with PAS. Positive rates with KONCPA were 77%, histology was 60%, KOH was 44%, and culture was 16%.[106] In another study, positive rates for fungal culture, KOH, calcofluor white staining, and KONCPA were 74.2%, 85.1%, 91.09%, and 99.01%, respectively. KONCPA also had the highest sensitivity and negative predictive value compared with the other methods.[107] A study was also performed on 493 patients with onychodystrophy who were evaluated by KOH, fungal culture, and PAS. PAS with KOH had the highest sensitivity (96%), followed by PAS and fungal culture (94%). The sensitivities for PAS, KOH and fungal culture, KOH, and fungal culture alone were 88%, 72%, 56%, and 29%, respectively.[108] A suggested algorithm is shown in Fig 15. Based on its low cost and rapidity, **[F15-4/C].** we recommend KOH with direct microscopy as the first diagnostic test for suspected cases of onychomycosis. If positive, fungal culture or PCR are ordered to identify the causative organism. If KOH testing is negative, it may be repeated to increase sensitivity[85] or, alternatively, PAS or PCR may be performed, which are more sensitive. If either PAS or PCR testing is positive, a fungal culture is performed. If results are discordant (ie, PCR is positive and culture is negative) or if there is evidence of Candida or NDM, both tests should be repeated with new samples.

FUTURE DIRECTIONS

Key points

- **Confocal microscopy and optical coherence tomography are emerging techniques for the confirmation of onychomycosis**
- **Limitations include specialized training and cost**

Confocal microscopy

Reflectance confocal microscopy uses a 830-nm laser in reflectance mode to generate horizontal sections in different depths between the nail plate surface and the nail bed. The resulting in vivo images can then be used to determine the presence or absence of hyphae (Fig 16).[109] Based on 2 clinical trials, the sensitivity was 58% to 80% and the specificity was 81% to 91%.[110,111] Limitations include an inability to confirm organism identity, limited depth of view (200 to 300 μm), specialized training requirement, and cost.

Optical coherence tomography

Optical coherence tomography uses low-coherence light to noninvasively image tissue with a depth of 1 to 2 mm.[112] In a pilot study using optical coherence tomography on 10 patients with histologically confirmed onychomycosis, fungal elements were

seen in all patients. The fungi appeared as elongated structures within the nail plate, while the surrounding areas were more homogeneous and exhibited decreased signal intensity.[113] In 1 study of 60 patients with onychodystrophy, optical coherence tomography had a sensitivity of 92% and a specificity of 43% for onychomycosis.[110] A disadvantage of this technique is a high number of false positive results because of the poor resolution, which does not allow distinction between hyphae and spores with air bubbles and other artifacts. Other limitations are the high cost of the device and the skill needed to optimize its use.

Other techniques

Other techniques that are being explored for the diagnosis of onychomycosis include infrared thermography,[114,115] flow cytometry,[116,117] immunochromatography,[118] and matrix-assisted laser desorption ionization—time of flight mass spectrometry.[119-121]

In conclusion, patients with onychomycosis may experience pain and difficulty with activities of daily living and onychomycosis has a negative impact on quality of life. While onychomycosis accounts for the majority of nail disorders seen in clinical practice, the physical examination findings are variable, and it can be mistaken for other benign and malignant nail conditions. Therefore, diagnostic confirmation is imperative and may be performed using direct microscopy, fungal culture, histopathology, PCR, or a combination of these techniques.

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Answers to CME examination

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