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REVIEW

EVOLVING ROLE OF NONDERMATOPHYTES IN ONYCHOMYCOSIS

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Onychomycosis is the term given to any fungal infection of the nail and represents one of the more common superficial fungal infections.[1] Greater medical attention needs to be placed on the identification, evaluation, and management of the pathogens that underlie this disease.

There is increasing awareness of nail infections. Also, nonpathogenic fungi, not normally associated with nail disease, may be emerging as pathogens by taking advantage of the impaired resistance of many of today's patients. In particular, a number of species of yeast in the genus *Candida* have been described as the etiologic agents of dystrophic nails and cases of nondermatophytic molds have been documented as nail pathogens. Likewise, the increasing frequency of mixed infections, from which dermatophytes, yeasts, and/or molds, or a combination thereof have been isolated, has important implications for the future management of onychomycosis;[2–7] however, one should remember, that the mere isolation of an organism from an infection site is not proof that it is the causative pathogen. Thus, although for many years yeasts and molds have been cultured from nails, they have been considered either contaminants or commensals and, therefore, ignored as the etiology of nail disease. A positive potassium hydroxide (KOH) preparation, showing hyphae, pseudohyphae, or yeast, is needed to prove a fungal etiology. Histopathological examination showing invasion of the fungus in a nail plate will positively confirm the etiology. Accumulating diagnostic evidence suggests that nondermatophytes can no longer be discounted as a potential cause of nail disease.

DIAGNOSIS

Until recently, because the etiology of cutaneous fungal infections was thought to be limited to dermatophytes, saprophytic organisms were not recognized as primary pathogens; these organisms were thought to be secondary to dermatophytic infections. Furthermore, clinicians were not motivated to confirm the clinical diagnosis with appropriate laboratory testing; however, recent studies have shown that clinical diagnosis alone is often inaccurate. In one large-scale study, involving 2750 cases of onychopathy, 70% were diagnosed clinically as fungal infections; of these, only 40% could be confirmed as fungal in origin by direct examination and culture.[8] A similar study at Louisiana State University found that the clinical diagnosis could not be confirmed in 57% of cases.[9]

Direct microscopic examination using a keratin-clearing agent, such as KOH or chlorazol black E, is necessary to detect the presence of fungal elements. Although more labor-intensive, histologic examination of the nail plate showing hyphal invasion provides definitive proof of a fungal etiology and may be necessary to confirm infections due to nondermatophytic agents. This is essential because of the high incidence of false-negative cultures. In one study, 28% of negative cultures were found to be positive on histologic examination.[8] In order to minimize false-negative results on culture, microscopic, and histologic examination, it is important that the debris be collected as proximal to the cuticle as possible. The debris from this area will have the highest number of fungal organisms.

The type of medium on which the isolate is grown can have an impact on the results, as well as limit the identification of the causative organism(s). Historically, cycloheximide has been used in dermatologic media to prevent the growth of nondermatophytes. Unfortunately, use of media containing cycloheximide inhibits the identification of many "saprophytes" that may be potential pathogens. These include some species of *Candida*, *Scopulariopsis brevicaulis*, *Scytalidium dimidiatum* (*Hendersonula toruloidea*), *S. hyalinum*, and some species of *Aspergillus*. It is imperative that the medical mycology reference laboratories culture all nail samples using both a cycloheximide-free medium (i.e., Sabouraud's agar), and cycloheximide-supplemented media,[10] such as dermatophyte test medium (DTM), Mycosel, or Mycobiotic agar (BBL, Baltimore, MD).

Gentamicin may be added to Sabouraud's agar to prevent bacterial overgrowth. The identification of a nondermatophyte mold or yeast as the pathogen re-

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quires stringent criteria.[11,12] The criteria are as follows: 1) absence of any dermatophyte on culture; 2) KOH positivity; showing the presence of filaments (hyphae), pseudohyphae, or yeast in the subungual keratin; and 3) pure culture; five cultural sites out of 20 must be positive for the nondermatophyte only.

These criteria eliminate the possibility of diagnosing "mixed infections" of the nail (i.e., that more than one organism may be the cause of the infection). This potential situation requires different techniques to analyze the infected nail.

In a Belgian laboratory, the technique of immunohistochemistry and flow cytometry was developed to study fungal nail dystrophy. This laboratory has unequivocally identified cases where different species of fungi are present in a nail plate.[13] In addition, the flow cytometry technique enables identification of individual genera and species of fungi.[14] These new methods are providing evidence that mixed infections do occur and, furthermore, that nondermatophytic molds and yeasts can invade the nail and cause primary infections in nails.[15]

ETIOLOGY

Evolving Shift of Pathogens

The etiology of onychomycosis, as well as that of other superficial fungal infections of the hair and skin, appears to be fluctuating. Factors, such as 1) the emergence of new organisms, 2) the variety of diagnostic techniques, 3) the definition of "what is a pathogen," and 4) the various geographic locations of the individual studies, all combine to influence the wide range of dermatophytes, yeasts, and molds that have been reported in the literature (Table 1). Although organisms vary according to the regional site,[1,16–20] dermatophytes account for the majority of fungi isolated as the causative pathogen in onychomycosis, ranging from 32[8] to 90.7%.[10]

Dermatophytes are far more likely to cause infections in toenails than fingernails.[3,8,18,19] By comparison, onychomycoses in the fingernails of women are usually caused by yeast infections.[8,17,18]

Table 1. Types of Fungi Found in Onychomycosis (%)

Study (Year)[Ref. No.]	*N*	*D*	*Y*	*N*	*Mixed*
Meinhof (1962)[25]	1844	44.3	29.2	15.5	11
Walshe and English[36]	373	56	33	11	NR
Achten and Wanet-Rouard (1978)[8]	1098	32	66	2	NR
Clayton (1992)[21]	669	81	17	2	NR
Greer (1993) unpublished	431	23	63	4	8

N = number of nails; D = dermatophyte; Y = yeast; N = nondermatophyte molds; NR = not reported.

Among the dermatophytes, *Trichophyton rubrum* is the most prevalent pathogen in both temperate and tropical climates.[3,8,10,17] Data were compiled from a routine diagnostic mycology laboratory in England. It was found that 462 of 539 (85%) dermatophytes isolated from nails were *T. rubrum*, followed by *Trichophyton mentagrophytes* var *interdigitale*, at 13%.[21] All data included both positive KOH preparations and culture. In another report,[3] also using data from both KOH and cultures, 3626 skin and nail samples were taken from 2507 patients. Dermatophyte infections were demonstrated in 700 patients (824 specimens), 77% of which involved the feet. The most common pathogen identified was *T. rubrum*, accounting for 66% of the dermatophyte infections. *T. mentagrophytes* was isolated from 33.2% of all patients and, as expected, was more frequently found in toenails than in fingernails.

Similarly, in a retrospective study over a 20-year period in the Netherlands,[22] 2079 superficial fungal infections were diagnosed. The relative incidence of dermatophytes was found to remain fairly constant at 66%; 315 (15%) of the infections were from nails, with *T. rubrum* and *T. mentagrophytes* the most prevalent dermatophytes isolated (57% and 18%, respectively). Interestingly, whereas *T. rubrum* was isolated predominantly in toenails (88%), *T. mentagrophytes* was isolated only in toenails. It is not surprising that the relative incidence of yeasts (and, in particular, *Candida albicans*) remained fairly constant at 25%. *Candida* species are normal flora of the human host. Lastly, it was noted that *Scopulariopsis brevicaulis* was the most frequent nondermatophytic mold causing infection of nails.[22]

Yeasts are often present in cutaneous mycoses,[2,17,21–23] especially in paronychial infections in women. In one report on paronychial infections, it was found that *Candida* spp, particularly *C. albicans*, were the cause of 79% of fingernail infections in women, but only of 22% of fingernail infections in men.[21]

In one study, using data from both KOH and culture,[8] yeasts were identified in 66% of onychomycoses; *C. albicans* was isolated in 72% of those cases. In a Canadian report that also combined KOH and culture data, over 4000 specimens of fungal infections of the nails, soles, and palms were studied.[10] Here, it was found that *C. albicans* was the third most prevalent organism from all sources, and the most predominant yeast isolated. Likewise, in another study of 10-years' duration, of 986 fungal nail infections in Western Australia,[17] *C. albicans* accounted for the majority of yeasts in fingernail infections (58.6%), but was less prevalent among toenail infections (17.1%). On the other hand, *Candida parapsilosis* was isolated in 40% of toenail infections and 24.6% of fingernail infections. Even though this yeast was the second most common fungus identified by Achten and Wanet-Rouard,[8] the significance of these isolates could not be determined.

It is now clear that nondermatophytic molds isolated from dystrophic nails[2,16,17,22,24] may be true pathogens. Onychomycosis caused by nondermatophytic molds, include *Scopulariopsis brevicaulis*,[2,3,21,25] *Aspergillus* spp,[2,3,16,17,21,22] *Scytalidium hyalinum*,[24] and *S. dimidiatum*. In fact, in one study, the third most common isolate was *S. brevicaulis*, which was identified in 233 of 3626 specimens (7%);[3] it was the fourth most common pathogen of the nail (1.6%) in yet another study.[10]

In 1987, the first case of *Hendersonula toruloidea* (*Scytalidium dimidiatum*) infection in the United States was reported.[26] Since then, additional cases have been reported in Europe[27-29] and elsewhere in the United States.[24,30] This dematiaceous fungus and a related hyaline species, *S. hyalinum*, are proven causes of onychomycosis, tinea pedis, and tinea manuum.[24] Documented cases have been reported predominantly from tropical and subtropical areas where this mold is endemic (i.e., the Caribbean, Africa, India, and the Far East).[31,32]

Mixed Infections

Another complicating aspect of onychomycosis is the prevalence of "mixed fungal infections," when more than one type of fungus is found within an infected nail. In the past, mixed infections went largely unrecognized because the tendency has been to ignore the nondermatophytes, whenever a dermatophyte was present. A number of studies have clinically evaluated the incidence of mixed infections (Table 2).[1,3,33]

It was found that, of 691 infected nails cultured (646 toenails and 45 fingernails), a single organism was isolated from 58% (23% dermatophyte, 24% yeast, 11% mold).[1] The other 42% of the cultures were polymicrobic (Fig. 1). Other reports do not support such a high incidence of mixed cultures; these ranged from almost 0[17] to 11%.[15] This variation reflects the difficulty of proving the etiology of such infections[2] in addition to the geographic area, sample types, and patients' variations in the different studies. For example, one report shows that toenails are more prone to infection with nondermatophytic species than fingernails, probably because of frequent trauma and the humid environment related to the daily wearing of shoes.[17] Reports confirm that nondermatophytic molds are more

Table 2. Mixed Fungal Infections (%)

Study	*D+D*	*D+Y*	*D+M*	*D+Y+M*	*Y+Y*	*Y+M*	*M+M*
Budak et al.[3] (N=1513)	6.4	4.7	7.4	9.1	14.8	33.9	23.7
Grigoriu et al.[33] (N=872)*	11	11.5	12.2	9.6	6.7	25	24
Willemsen[1] (N=691)	NR	23	6	9	NR	5	NR

D = Dermatophytes; M = Nondermatophyte molds; Y = Yeasts; NR = not reported, only toenails.

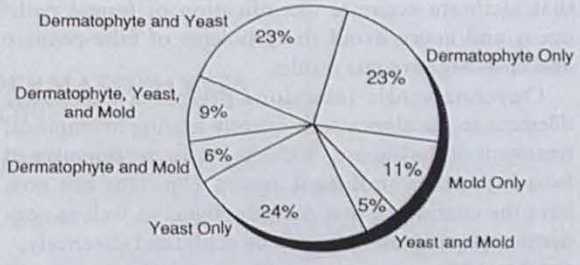

Figure 1. Willemsen—Incidence of mixed cultures in nails.

prevalent in nail infections in the hot and humid tropical and subtropical parts of the world.[13] Thus, the source of nail samples may influence the overall etiologic picture produced by studies of infected nails.

IMPLICATIONS FOR TREATMENT

The diversity of etiologic agents associated with onychomycosis has important implications for the management of fungal nail infections, a disease entity that has been notoriously difficult to treat. Griseofulvin has been the most commonly used oral antifungal treatment for onychomycoses; however, it is effective only against dermatophytic infections. Its high relapse rate may be due to infections caused by yeast and molds.[13]

Fortunately, recent advances have taken place in the antifungal armamentarium with the development of broader spectrum drugs, fluconazole, terbinafine, and itraconazole. Itraconazole, in particular, has activity against most fungal pathogens of the nail (dermatophytes, yeast, and molds) and has been associated with good clinical results;[5,34,35] however, no antifungal agent has yet to be found effective against *H. toruloidea*.[4,26,27,31]

CONCLUSIONS

Although dermatophytic fungi are still the main etiologic agents of onychomycoses, some species of nondermatophytic molds and yeasts are also capable of invading the nails. Reports in the current literature indicate that not only is the awareness of onychomycosis increasing, but nail diseases caused by nondermatophytes and mixed infections are becoming more prevalent. There is a need to change diagnostic laboratory procedures to account for the new evidence that nondermatophytes and yeasts are important in the etiology of onychomycoses. Specifically, this includes the consistent use of appropriate microscopic evaluation, in conjunction with the culturing of all specimens on both cycloheximide-free and cycloheximide-containing media. Histologic examination can be used to verify the invasion by a nondermatophyte fungus. In addition, histochemical techniques have been developed

that facilitate accurate identification of fungal pathogens and hence avoid the problems of false-positive and false-negative test results.

Onychomycotic infections present a diagnostic dilemma to the clinician, frequently leading to empirical treatment of the disease. With the recent development of broad-spectrum antifungal agents, clinicians can now have the confidence that dermatophytic, as well as non-dermatophytic pathogens will be eradicated effectively.

DRUG NAMES

fluconazole: Diflucon
griseofulvin: Fulvicin, Grifulvin V, Grisactin
itraconazole: Sporanox
terbinafine: Lamisil

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